การย้ายที่ของพลาสติไซเซอร์จากแผ่นปะเก็นรองฝาโลหะของขวดแก้วสู่อาหาร

นางสาวจันทร์จิรา จันทร์ประเสริฐ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

MIGRATION OF PLASTICIZERS FROM METAL LID GASKET OF GLASS JAR
INTO FOOD

Miss Chanchira Chanprasert

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2009

Thesis Title	MIGRATION OF PLASTICIZERS FROM METAL LID GASKET OF GLASS JAR INTO FOOD
By	Miss Chanchira Chanprasert
Field of Study	Chemistry
Thesis Advisor	Assistant Professor Natchanun Leepipatpiboon, Dr., rer.nat

---

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

S. Hannongbua
.... Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr., rer.nat)

THESIS COMMITTEE

Sirirat Kokpol
.... Chairman
(Associate Professor Sirirat Kokpol, Ph.D.)

Natchanun Leepipatpiboon
....Thesis Advisor
(Assistant Professor Natchanun Leepipatpiboon, Dr., rer.nat)

Putthi V.
.... Examiner
(Puttaruksa Varanusupakul, Ph.D.)

Panthira Ketkaew
.... External Examiner
(Assistant Professor Panthira Ketkaew, Ph.D.)

จันทร์จิรา จันทร์ประเสริฐ : การย้ายที่ของพลาสติไซเซอร์จากแผ่นปะเก็นรองฝาโลหะของขวดแก้วสู่อาหาร. (MIGRATION OF PLASTICIZERS FROM METAL LID OF GLASS JAR INTO FOOD) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.ณัฐชนัญ ลีพิพัฒน์ไพบูลย์., 111 หน้า.

การศึกษาการจำลองการย้ายที่ของพลาสติไซเซอร์จากฝาโลหะของขวดแก้วโดยพิจารณาจากตัวแปรต่างๆ ได้แก่ ระยะเวลาการสัมผัส อุณหภูมิที่สัมผัส ความร้อนเริ่มต้น และพื้นที่ผิวสัมผัส ในกรณีศึกษานี้ใช้ตัวอย่างฝาลักขนาด 48 มิลลิเมตร และ 63 มิลลิเมตร ทำการทดสอบคัดองค์ประกอบของปะเก็นเริ่มแรกด้วยเทคนิคแอทเทนนูเอทโททอลรีเฟลคชันฟูเรียทรานส์ฟอร์มอินฟราเรดไมโครสเปกโทรสโคปีและวิเคราะห์ด้วยเทคนิคแก๊สโครมาโทกราฟีเฟลมไอออไนเซชัน พบพลาสติไซเซอร์สี่ชนิด คือ อีพอกซีไดซอยบีนออยล์ ไตรอะซิทิน ไดบิวทิลเซบาเคต และไดไอโซโนนิลไซโคลเฮกเซนไดคาร์บอกซีเลท ปริมาณรวมของพลาสติไซเซอร์ที่พบในฝาลักขนาด 48 มิลลิเมตร และ63 มิลลิเมตร เท่ากับ 540.2 ± 14.1 มิลลิกรัม และ 924.5 ± 105.5 มิลลิกรัม ตามลำดับ การศึกษาการย้ายที่ของพลาสติไซเซอร์ทำตามวิธีมาตรฐานของสหภาพยุโรปโดยใช้น้ำมันมะกอกเป็นตัวแทนอาหารประเภทน้ำมัน พบว่าขนาดพื้นที่ผิวสัมผัสเป็นปัจจัยหลักที่ก่อให้เกิดการปนเปื้อนตามด้วยอุณหภูมิที่สัมผัสและระยะเวลาการสัมผัส ส่วนกระบวนการให้ความร้อนเริ่มต้นไม่มีผลต่อการย้ายที่ของพลาสติไซเซอร์ การย้ายที่ของพลาสติไซเซอร์ในระหว่างกระบวนการเก็บรักษายังขึ้นอยู่กับขนาดและรูปร่างโมเลกุลของสารนั้น พลาสติไซเซอร์ที่มีขนาดโมเลกุลเล็กและมีโครงสร้างเป็นเส้นตรงส่งผลต่อการย้ายที่เป็นอย่างมาก

ภาควิชา เคมี ลายมือชื่อนิสิต จันทร์จิรา จันทร์ประเสริฐ
สาขาวิชา เคมี ลายมือชื่ออ.ที่ปรึกษา ณัฐชนัญ ลีพิพัฒน์ไพบูลย์
ปีการศึกษา 2552

4972245223 : MAJOR CHEMISTRY
KEYWORDS : MIGRATION / PLASTICIZERS / GASKET / METAL CAP

CHANCHIRA CHANPRASERT : MIGRATION OF PLASTICIZERS FROM METAL LID GASKET OF GLASS JAR INTO FOOD.
THESIS ADVISOR : ASST. PROF.NATCHANUN LEEPIPATPIBOON, Dr.rer.nat., 111 pp.

Simulation of plasticizer migration from jar metal caps was studied. The parameters investigated are contact time, contact temperature, initial heating, and contact surface area. The study was conducted on lug caps size 48 mm and 63 mm. The gasket composition was first screened by Attenuated Total Reflection Fourier Transform-infrared microspectroscopy and analyzed by gas chromatography flame ionization detector. The data identified four types of plasticizers: ESBO, TAC, DBS and DINCH. Total plasticizer contents were 540.2 ± 14.1 mg and 924.5 ± 105.5 mg per cap size 48 mm and 63 mm, respectively. The migration study was performed according to the European Union standard method using olive oil to represent fatty foods. The simulated migration data identified contact surface area as the primary source of contamination followed by contact temperature and contact time. Initial process heating has no influence on the migration. Migration sources during storage were size and molecular shape of the plasticizers. Small linear plasticizer contributed largely to the total migration.

Department :	Chemistry	Student's Signature	Chanchira Chanprasert.
Field of Study :	Chemistry	Advisor's Signature	
Academic Year :	2009		

ACKNOWLEDGEMENTS

First of all, I would like to express my deepest gratitude to my thesis advisor, Assistant Professor Dr. Natchanun Leepipatpiboon, for her professionalism, discerning suggestion, forbearance, encouragement, continuous assistance and critical proofreading throughout of this research. I would like to extend my thanks to Assistant Professor M.L. Siripastr Jayanta, for her plentiful guidance and a kind patience for my mistake and critical reading. Special thanks Associate Professor Dr. Sanong Ekgasit and all members in Sensor Research Unit (SRU) for ATR FT-IR microspectroscopy support and their valuable recommendations and generosity.

I wish to thank the Department of Chemistry, Chulalongkorn University, for all facilities and partial grant. Furthermore, I would like to thank the innovations for the Improvement of Food Safety and Food Quality for New World Economy and Center for Petroleum, Petrochemicals and Advanced Materials for their financially supports. Special thanks Mrs. Sumalee Tangpitayakul and her colleagues of the Department of Science Service, Ministry of Science and Technology for GC-MS support and information in this work.

In addition, I would like to thanks all members in Chromatography and Separation Research Unit (ChSRU) for their suggestions. I also specially thanks to all my friends of Chulalongkorn University for their helpfulness and heartfelt friendship. Thanks are also extended to everyone who has contributed suggestions and supports throughout this research.

Finally, I am eternally grateful to my beloved parent, my brother and my sister for their unconditional love, understand, cheer and enormously ever-willing support to the end.

CONTENTS

PAGE

CHAPTER I : INTRODUCTION

CHAPTER II : THEORY

CONTENTS (continued)

PAGE

CHAPTER III : EXPERIMENTAL

CHAPTER IV : RESULTS AND DISCUSSION

CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

FIGURES **PAGE**

LIST OF FIGURES (continued)

FIGURES **PAGE**

LIST OF FIGURES (continued)

LIST OF ABBREVIATIONS

acPG	acetylated partial glyceride
ATBC	acetyl-*tri*-butyl citrate
ATR	Attenuated total reflection
C20:2	diepoxy eicosadienoate
D	diffusion coefficient
Da.	Dalton
DBS	dibutyl sebacate
DEHA	di-(2-ethelhexyl) adipate
DEHP	di-(2-ethelhexyl) phthalate
DIDP	diisodecyl phthalate
DINCH	diisononyl cyclohexane-1, 2-dicarboxylate
DINP	diisononyl phthalate
DHP	diheptyl phthalate
DMP	dimethyl pimelate
DPS	dipropyl sebacate
EA	erucamide
EN	ethyl naphthalene
ESBO	epoxidized soy bean oil
ETP	electrolytic tinplate
FID	flame ionization detector
FT-IR	Fourier transform-infrared spectroscopy
GC	gas chromatography
Ge	germanium
HPLC	High Performance Liquid Chromatography
IRE	internal reflection element
IS.	internal standard
K	partition coefficient
KBr	potassium bromide
MCT	Mercury-Cadmium-Telluride
mL	milliter

mm	millimeter
MS	mass spectrometry
M.W.	molecular weight
NPLC	Normal Phase Liquid Chromatography
OA	oleamide
PAs	polyadipates
PTV	programmed temperature vaporizing
PVC	poly(vinyl chloride)
RF	response factor
TAC	triacetin
TFS	tin-free steel
THF	tetrahydrofuran
TIR	total internal reflection
µm	micrometer
µ	micro
n	refractive index
T_g	glass transition temperature

CHAPTER I
INTRODUCTION

1.1 Problem Definition

Food packaging plays an important role in canned food and food processing. The functions of food packaging are to hermetically seal to prevent the entry of contaminants, microorganism and to protect the content against damage during transporting, storage, and distribution. Furthermore, the design of food packaging can be used to attract the consumer’s attention. However, the packaging of food products must not release chemicals from its material to avoid a negative impact on the quality and safety of food as well as to prevent potential health damage from consumers.

Plastic material is mainly applied for food packaging, especially polyvinyl chloride (PVC). It is one of the most versatile plastics and provides a good resistance to light, oil, and chemicals. PVC is a thermoplastic obtained by the addition polymerization of the vinyl chloride monomer. Depending on its application, plasticizers are mixed in the PVC processing in order to improve the properties of the plastics.

In addition, glass jars with metal lids are widely used for packed food. To reduce the friction between the rim of glass jar and the metal lid, the inner part of the metal lid is lined with a gasket against the rim of glass jar. Moreover, this provides tightness to easily open and close the lid. The gasket consists of PVC plastisols containing 25-45% additives by weight. This is the maximum amount of plasticizers used. On the other hand, gaskets are composed of stabilizers, slip agents, lubricants, and blowing agents.

As the gasket is direct contact with the food content in the glass jar as showed in APPENDIX A, the migration of the plasticizers from the gasket into the food appears to be possible. This migration may occur during the processing of food i.e., packaging, transport, storage, and distribution. The plasticizers/additives transfer from the gasket into foods can possibly cause damage to consumer health.

For over twenty years, problems of the migration of plasticizers/additives were observed. The contamination of the migrants in the food may far exceed the European Union (EU) legal limits, which are regulated by the overall migration limit (OML) of 60 mg/kg. The most abundant problems caused by the contact between the oil or fat food product and the gasket on the metal lid can be observed for oily sauces, products immersed in oil, pesto, infant foods, and preserved herb in oil. The migration of plasticizers depends on the polymer properties, the nature of plasticizers (e.g., structure, molecular weight/viscosity, polarity), the plasticization, the homogeneity of the compound, as well as the contact time and temperature.

The key reason for studying the migration of plasticizers from gasket into food content is the potential diverse health effects on the consumers from the exposure to these components. The demonstrated carcinogenicity in rodents, the potential estrogenic effects, and the reproductive effects on humans have revealed the toxicology of several commonly used plasticizers. On the other hand, the migration of these toxicological components may have an additional effect on the commerce as they may be restricted or even banned.

1.2 Regulatory Standard in the European Union

The materials used as plastics in food industry are regulated on EU level in the Directive 2002/72/EC [1], Directive 2004/19/EC [2] (amended Directive 2002/72/EC), and Directive 2007/19/EC (4th amended Directive 2002/72/EC) [3]. Directive 372/2007/EC legislated for transitional migration limits for plasticizers in the gasket of the lids that come in contact with the foods are given [4].

The specific migration limits (SML) of plasticizers in gaskets are regulated as follows: Epoxidized soybean oil (ESBO) is authorized as an additive in plastics for foods with restriction of 60 mg/kg SML for general foods and 30 mg/kg SML for infant foods [1, 3 – 4]. As phthalates may be released from many source and are highly toxic, the SMLs are lower, i.e., SML of 3 mg/kg for di-(2-ethylhexyl) phthalate (DEHP) [3]. For diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP) are summarized as a group-SML of 9 mg/kg [1]. For acetyl-*tri*-butyl citrate (ATBC), the

SML is 60 mg/kg in food or food simulant [4]. For di-(2-ethylhexyl) adipate (DEHA), the SML is 18 mg/kg and for the polyester of adipic acid with unbranching of C_{12} – C_{22}, the SML is 30 mg/kg (with M.W. < 1000 Da). For oleamide and erucamide, SMLs of 0.05 and 30 mg/kg are regulated, respectively. Diisononyl-cyclohexane-1,2-dicarboxyate (DINCH) is a rather new plasticizers; therefore, there it has not been regulated by the EU, but in Germany, a SML of 5 mg/kg is approved [1, 4].

1.3 Literature Review

a) Literature review on *The analysis composition of plasticizers in gasket*:

Biedermann-Brem *et al* [5] studied the composition of gaskets and its potential migration into food. The variety of the additives in PVC gaskets were found as 25-45% (w/w) of additives including plasticizers, stabilizer, slip agent, and lubricants. Among the most commonly detected compounds in PVC gaskets were small molecular weight compounds, such as phthalates, adipates, sebacates, acetyl-*tri*-butyl citrate, as well as compounds of large molecular weight as triglycerides like epoxidized soybean oil or polyadipates (mixture compounds of adipic acid) [6 – 10].

Fantoni and co-workers [11] and Pedersen *et al* [10] studied the spectra of the composition in gaskets. The qualitative analysis showed ESBO and phthalates as principle components, but also amides as slip agent in the gaskets.

b) Literature review on *The migration of plasticizers from gasket into foods*:

Hammarling *et al* [12] reported the contamination of ESBO in baby foods with level ranges from <1.5 to 50.8 mg/kg. In addition, for baby foods, which had never been in contact with the gasket, no ESBO could be detected. Therefore, the migration of ESBO can be attributed to the contact of the food with the gasket. Furthermore, in a European survey of contamination of baby food, ESBO was found in 95 of 248 samples in levels of 1.5-135.2 mg/kg. 15% of samples exceeded 30 mg/kg and 4 % exceeded the OML of 60 mg/kg [11, 13].

Fankhauser-Noti *et al* [13] studied the migration of ESBO with consideration of ESBO contents in the gasket and the modes of contact. For the ESBO in the gasket it was found that there was no obvious relationship between the weight of the gasket in contact with the food and the diameter of the lids referring to the same size of the lids. On the other hand, the difference in weight reflected the amount of ESBO transferred into the food. The modes in which food is in contact with the gasket can be described as follows: the first contact in which the oil layer of food is adhering to the gasket and extracting the ESBO in the gasket. Then, during the next step, the oil layer adhering to the gasket that contains the extracted ESBO is replaced by a new fresh oil layer and the extracted ESBO is transferred into the food in the jar. Subsequently, this process is repeated. It has been shown that this process mainly affects the migration of ESBO as for food of a firm consistence, less migration is observed than for food with free oil on the surface.

Suman *et al* [14] developed an analytical method using reverse-phase HPLC – MS/MS for the determination of ESBO in food. For ESBO determined in a variety of food in different matrices, the concentration of detected ESBO covered a range of 10 to 100 mg/kg. The variable ESBO content that was found obviously indicated a concern for the nature and fat content or dispersion of the food matrix.

Fankhauser-Noti and co-workers [8] investigated the migration in terms of the proportion of the amount present in the gasket compared to that directly in contact with the food. The migration of small plasticizers (e.g., DEHA, ATBC, DINP, DIDP) far exceeded 100%, which may have been because the parts underneath and outside of the jar rim were included in the investigation. Hence, the migration depended on the diffusion of plasticizers, while small plasticizers showed a high diffusion through the ESBO.

Fankhauser-Noti *et al* [6] collected oily products in glass jars directly from the distributor and ethnic shops. In their survey, they found concentration of 1,170 mg/kg of ESBO including DIDP, DINP, DEHP, and DEHA, which were far exceeding SMLs. In consequence; this has shown the problem about the migration from the gasket into food. Some of the compounds found have not been solved since 1998; on the other hand, new plasticizers were found which have not been authorized by the EU such as DINCH, Ehol-16, and Ehol-18.

Pedersen *et al* [10] studied the level of several plasticizers migrating into fatty foods packed in glass jars with twist closures and determining the OML of lids. The amount of plasticizers migrated in high fat content was higher compared to the lower fat content. The migration of phthalates far exceeded the migration of ESBO. In addition, the parameters affecting the migration of plasticizers were found to be the area and thickness of gasket, the lid surface to sample ratio, the applied procedure of sterilization/pasteurization, the consistency of foods, and the handling of the jar. The OML of the lids into the food simulant olive oil was determined at 40 °C for 10 days. It was found that the OML was far exceeding 60 mg/kg; thus, the products were rejected from the market.

c) Literature review on *The migration test in food simulants*:

Migration testing is usually performed using food simulants rather than real foods. The complexity of foods and their analyses have many drawbacks in both analytical and practical aspect. Therefore, food simulants have been chosen as models for various types of foods (aqueous, acidic, alcohol, and fatty foods) as simplified migration replication.

Fankhauser-Noti *et al* [8] investigated the migration of plasticizers from the gasket into fatty foods simulant (olive oil). The migration test was studied under sterilization condition, the olive oil was heated to 120 °C for 1 hour followed by keeping the closed jar upside down at 40 °C for 10 days. The migration exceeded the average observed in real products.

Pedersen *et al* [10] studied the overall migration of unused lid with glass jar using olive oil as food simulants. The heated olive oil was filled into the glass jars, the lids were exposed the olive oil by turnover the samples and kept at 40 °C for 10 days. The results showed far exceeding OML of 60 mg/kg and the products were drawback from the market.

Biedermann *et al* [15] studied the migration of plasticizers from the gasket of lids under simulation conditions. The migration was studied for three types of gaskets: gasket with exclusively ESBO, gasket with exclusively PAs, and the gasket that consisted of ESBO, PAs, DBS, and EA as plasticizers. Conventional migration testing was performed under pasteurization and non-pasteurization, converting them at 40 °C for 10 days. The main parameters to have an influence on the migration were the characteristics of the oil, the initial heating, and the amount of oil in contact with the gasket. The oil properties were investigated; the oil was composed of small triglycerides, which easily penetrate the PVC and render the gasket making it more permeable for the migration of plasticizers. The initial heating had a strong impact on the migration; at the lower temperature the migration occurred slowly and the plasticizers from below were enabled to refill at the surface causing a continuous migration. At higher temperature, the plasticizers form a layer hindering the migration and causing the migration to be temporarily blocked until the next contact. Depending on the amount of oil in contact, the plasticizers migrated into food simulant. In addition, a weight loss of lids could be related with the oil entering in the gasket.

d) Literature review on *The Analytical procedures:*

Biedermann-Brem *et al* [5] determined plasticizers in gaskets by GC-FID; several plasticizers in the gaskets were analyzed at the same time with this easily detectable technique. The extract solution of the PVC gasket was analyzed, the commonly small plasticizers could directly be analyzed, whereas the large molecular plasticizers like ESBO or PAs had to be transesterified to smaller methyl ester molecules prior to analysis. The results of the transesterification reaction from the same extract solution confirmed the plasticizers observed in direct analysis [11, 13].

A spectroscopic technique, namely FI-IR spectroscopy was used as a screening method for the identification of plasticizers in the gasket [11] with spectral transmission by an KBr pellet of the extracted sample, and ATR FT-IR spectroscopy for the *in situ* determination by ATR accessory with diamond [10, 11]. The existence of characteristic bands of CH_2 at 2852/2885 cm^{-1} and 2921/2926 cm^{-1}, binding of –CO at 1740/1744 cm^{-1} and 1660, 1633 cm^{-1} indicated the presence of phthalates. The wavenumber of 3359/3361 cm^{-1} and 3188/3192 cm^{-1} implied breaking bonds of epoxide in ESBO, while N-H bonds were indicated at 3188/3192 cm^{-1}.

Several methods were described in the literatures for analysis of plasticizers in foods or food simulants using chromatographic techniques. Castle *et al* [16] pioneered the method for ESBO analysis by GC-MS. The analysis was based on determining methyl esters of diepoxy linoleic acid, using 5% phenyl methyl polysiloxane as separation column. The lipids were extracted from food and subsequently transesterified with sodium methoxide. The methyl esters were derivatized to form 1,3-dioxolane derivate by trimethylpentane and cyclopentanone [11, 12].

The analysis of ESBO is mostly published using on-line NPLC-GC-FID by using cyano-derivatized silica gel serves to isolated the diepoxy linoleic acid (analyte) with MTBE/pentane as mobile phase; the liquid driven by the LC pump to transfer the fraction of analyte to GC involved concurrent solvent evaporation. The separation column was 50% cyanopropyl-phenyl and 50% methylpolysiloxane (PS-225). Fankhauser-Noti *et al.* proposed on-line NPLC-GC-FID as an automate technique, suitable for the routine analysis without extraction of lipid and the formation of dioxolanes, which eliminated the sample preparation step. The lipids were transesterified from the homogenized sample food and injected as solute into NPLC [6, 8, 9, 10, 13].

Biedermann-Brem *et al* [17] developed an analytical method using GC-FID for the detection of ESBO in oily food samples, using a polar column of cyanopropyl polysiloxane. The quantity of ESBO found ranged between 36-374 mg/kg and the detection limit was lower than 20 mg/kg, whereas the detection limit of GC-MS is than necessary for this purpose.

The analysis of other plasticizers such as phthalates, adipates, ATBC or slip agent such as OA or EA involved GC-MS with injector-internal thermal desorption, which enables the detection of a wide range of plasticizers with a minimum sample preparation [18 - 19]. Diluted oil sample was injected onto the plugs of glass wool at the bottom of the liner wall, the solutes were volatilized and transferred to the separation in the splitless mode, while a lower solutes volatility required higher injector temperature of above 200 °C to clean the column inlet and to achieve a backflushing after the analytes were transferred to the separation column. High boiling bulk material (oil) was removed from the injector through the split outlet [8, 10]. The PTV injection provided an injection with variable injector temperature [20]. The PTV injector was composed of three steps: (i) deposition of the sample onto a short plug of glass wool with a cone-shaped syringe needle, a low initial temperature of the injector of 90 °C prevented the sample from evaporating inside the syringe needle, (ii) desorption of solutes as a sequence of elevated temperature as at high temperatures the solutes volatilize into the separation column, (iii) heating-out of the oil; the temperature was elevated to 350 °C to evaporate the triglycerides from the injector through the split outlet [6].

1.4 Hypothesis

In general, oily or fatty foods are packed in glass jar with metal lid included with PVC gaskets. PVC gaskets have an oil-resistant character and sponge-like structures with high amounts of plasticizers render the hermetic seals. As plasticizers are incorporate in gaskets and most of them are of lipophilic character, the plasticizers potentially migrate into oily food contents [21]. Moreover, the migration is a rather complicate process as it is related to the fat content of the foods, the consistency of foods, the contact area of the gasket with the foods, the structure of plasticizers in the gasket, and the initial heating. Thus, in this study, it was tried to simulate conditions to study how the parameters affect the migration of plasticizers from gasket into fatty food simulant (olive oil) instead packed food. As usually food is rather complex mixture, the migration testing in this study used food simulants. Additionally, most of plasticizers were ester compounds; therefore, the analysis was performed by GC analysis.

1.5 Purpose of the Study

As described in the literature review, many authors have studied the migration of plasticizers into food in glass jar with metal lid. There have are been many reports on the contamination of plasticizers and additives in the food content from the gasket, which lead to the suggestion of bad impact to the consumer’s health. In addition, the reports of migration testing in fatty food simulants (olive oil) showed the concern of the migration according to standard conditions (pasteurization/sterilization followed by 40 °C for 10 days) observed in reality. Up to now, no migration testing has been performed to study the parameters, which affect the migration of plasticizers.

It was intended to study the parameters that affect the plasticizer’s potential migration into fatty food simulant (olive oil) by simulation. The studied parameters included the contact time, the contact temperature, the initial heating, and surface contact area of gasket. The simulation started with an investigation of plasticizers consisting in the gasket as this is the source of the migration to the foods. Commonly used GC-FID was compared with the developed ATR FT-IR microspectroscopy because this is the technique without sample preparation step. In next step, plasticizers in fatty food simulant were determined according to each of the simulated conditions.

CHAPTER II
THEORY

2.1 Glass Jar and Metal Lid

A glass jar is one type of glass containers, which is used for canned food. The apparent characteristic is that its transparency and visibility of the product it contains is attractive to the consumer. Commonly, a glass jar comprises of two separate elements i.e., glass container and metal closure.

2.1.1 Glass Container

The glass container is an ideal container material for canned food. They mainly consist of soda-lime glass produced by a blowing and pressing technique; hence, it is a chemically stable and inert material that withstands the high temperatures in the canning process and the non-additives in the packaging. Additionally, it does not support or facilitate microbial growth on its surface and is impermeable to gases, liquid, bacteria, and odors [22 – 23]. A glass jar is a cylindrical container with a wide mouth and can be divided into three parts as follows (Figure 2.1):

1. **The finish:** the part of the jar that holds the cap, which is designed specifically for hermetically sealed enclosure. The appropriate finish depends on the type of closure during food canning process.
2. **The body:** the largest part of the container, which is filled with the food; a variety of shapes and sizes ranging from capacities of a few milliliters to several liters.
3. **The bottom:** the plate part, which is matching the body-mold; this part is specially designed to interlock the bottom of the jar with the closure of another jar for vertically stacking during handling or transportation.

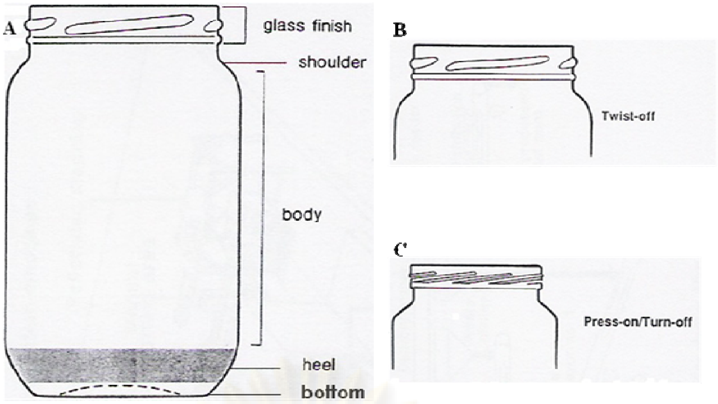


Figure 2.1 (A) The basic parts of a glass jar, (B) The glass finish of twist-off and (C) The glass finish of press-on twist-off, respectively.

2.1.2 Metal Lid Manufacturing

The metal lid of the glass jar is made of tin-plated (steel coated with tin) or tin-free steel (steel coated with chromium and chromium oxide) (TFS) [24]. The metal lid manufacturing is shown in Figure 2.2. A coil of metal is cut into sheets of convenient size for the printing and lacquering machines. The metal sheets are coated with printing inks outside of the sheet and lacquer to its inner surface to protect the metal of the lid from corrosion from the content of the glass jar. The printed and lacquered metal sheets are fed into stamping presses, which cut them into disks. These disks are pressed and curled into shape, while the shell provides a wide annular channel and the edges are rolled to give a smooth edge and then rolled again to form lugs. The liquid lining compounds are based on thermoplastic PVC resin called plastisol and are injected into the annular channel whilst rapid rotating. The rotary action serves to flow the lining compound into channel. A final heat treatment in an oven is done in order to cure and set the compounds at around 200 °C for 90 seconds to form a gasket around the periphery inside of the metal lid [22, 25].

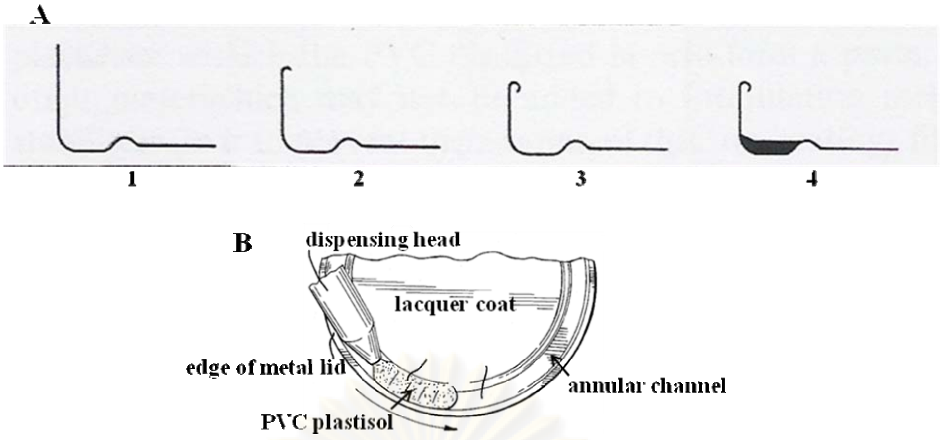


Figure 2.2 (A) The manufacturing of metal lids (1 – 4) and (B) The plastisol lining metal lid.

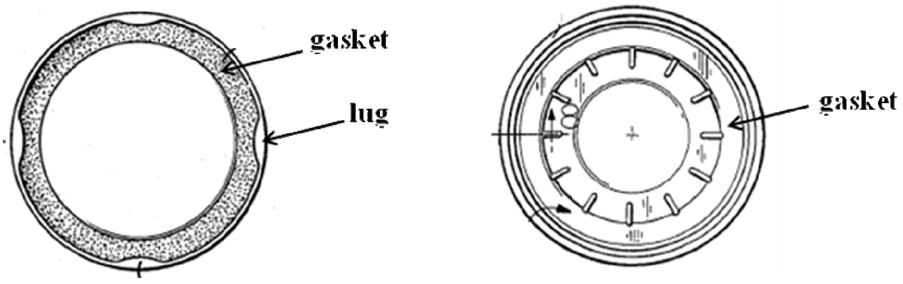
Typically, there are two types of metal lids used for glass jars (Figure 2.3):

2.1.2.1 Twist-off or Lug Cap

The twist-off or lug cap is made of tin-free steel (TFS) or tin-coated steel plates. This cap is capable of withstanding post-pack processing including retorting, sterilization or pasteurization. The finish of glass container exhibits an appropriate design corresponding to the internal lug of the cap. Lug caps are used in a wide range of products such as fruit juices, sauces, jams, pickled, product flavors, or food products that require vacuum for preservation.

2.1.2.2 Press-on Twist-Off Cap

The press-on twist-off cap is made of TFS or electrolytic tinplate (ETP) steel plate. The cap does not use thread or lugs to hold its cap on the glass container; this cap is pressed on a glass finished to seal and twisted to open. This cap is widely used to hot-filled and cool-filled products such as infant foods, vegetables, juices, fruits, and sauces.



A) Twist-off or Lug cap B) Press-on Twist-off cap

Figure 2.3 The metal lids used for the glass jar: (A) twist-off or lug cap, (B) press-on twist-off

2.2 Additives in PVC Gaskets

The gaskets of the glass jars are made of plastisol consisting of PVC with 25-45% by weight of additives, a majority of additives in PVC gasket as plasticizers. The gasket have a soft and sponge-like structure in order to hermetically seal, reduce the friction between jar and lid, and render the lid to easily open and tightly close [13].

Plasticizers are substances that are added to plastics in order to improve processibility and flexibility or stretchability by decreasing the melt viscosity, glass transition temperature (T_g), and modulus of elasticity without alteration of the chemical character of the polymer. Plasticizers are embedded between the chains of polymer by van der Waals force; then, increasing the free volume and lowering T_g for the plastics and softening it. Pure PVC is fairly unstable and easily degrades through heat, light, and mechanical energy; therefore, plasticizers are of major importance for the processing of PVC [21].

Apart from plasticizers, stabilizers are the most abundantly used additives in plastic. Generally, ESBO are widely used in food contact plastics heat stabilizers, lubricants and plasticizers as well. ESBO serve as a scavenger for hydrochloric acid liberated form PVC during the heat treatment for curing the gaskets [26 – 27] as shown in Figure 2.4.

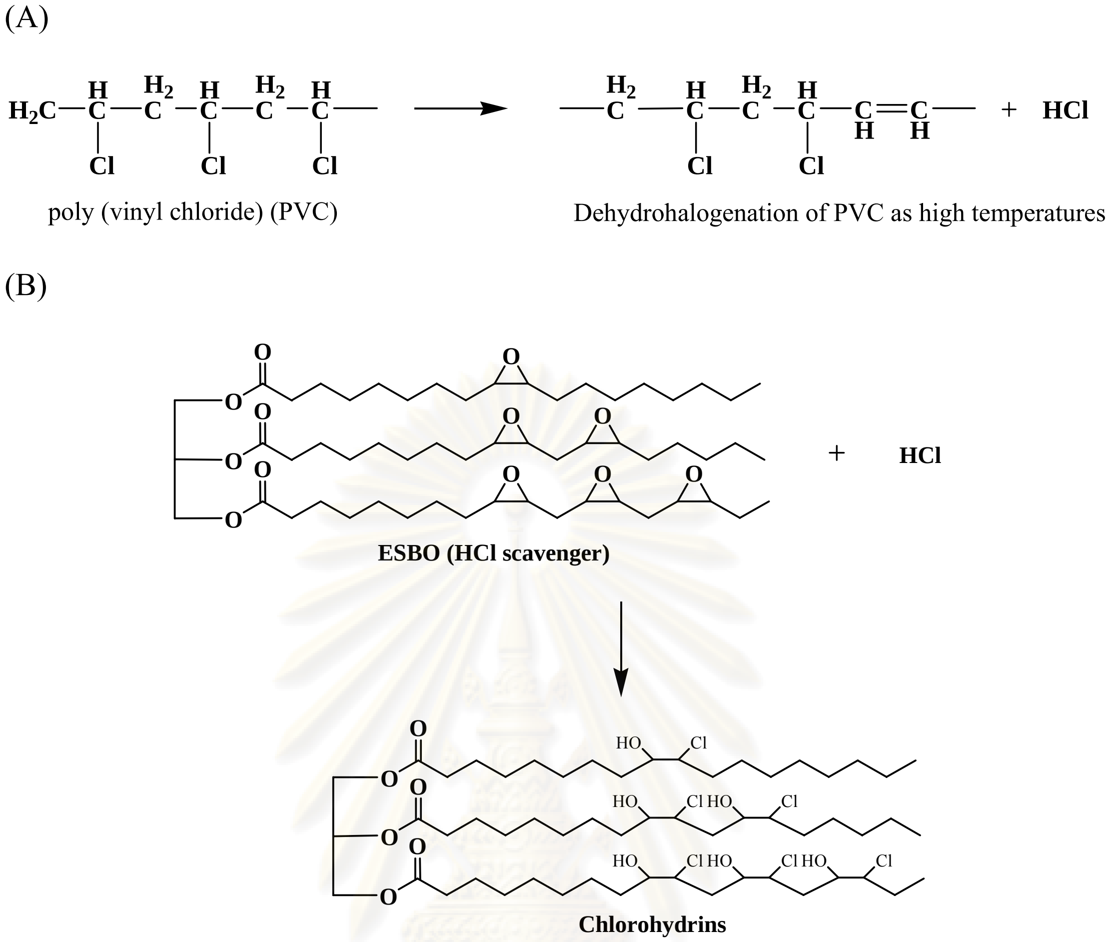


Figure 2.4 (A) Degradation of PVC at heat treatment process for curing gasket and (B) ESBO reacts with HCl results in chlorohydrins.

Furthermore, slipping agents such as OA and EA are also added to plastic formulations where they gradually tend to bloom to the surface imparting useful properties including lubricants, prevention of film stick together and reduction of static charge [27].

2.2.1 Structure and Chemical Properties

There are many types of additive used in PVC gasket such as plasticizers, stabilizers and slipping agent, as showed in Table 2.1. Furthermore, plasticizers/additives were incorporated in PVC gaskets as seals potentially migrate into the food during sterilization and storage [5-8].

Table 2.1 The chemicals properties of additives used in PVC gasket.

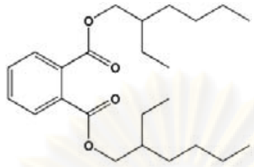
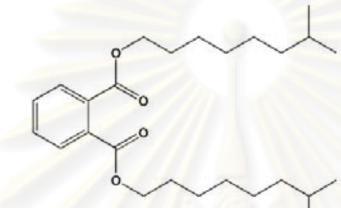
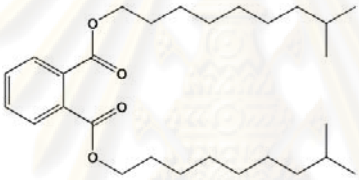
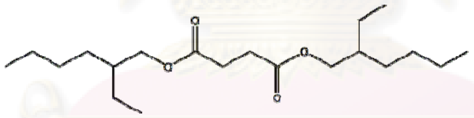
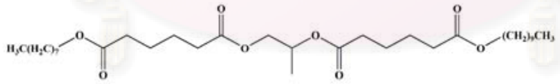
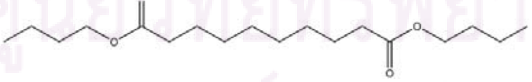
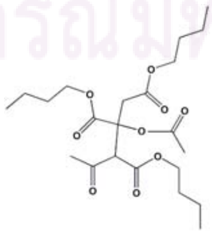
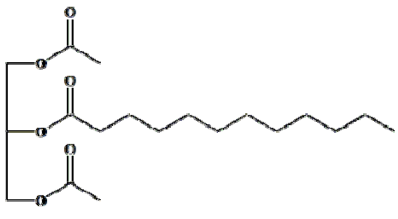
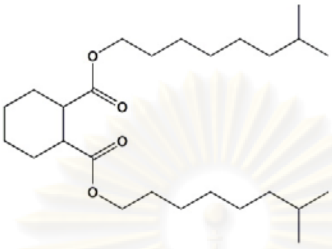
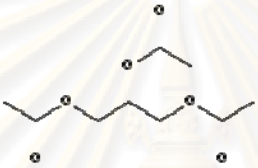
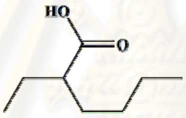
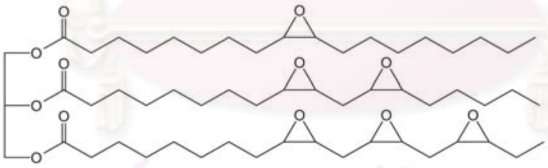
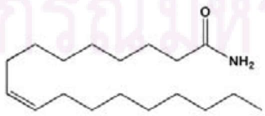
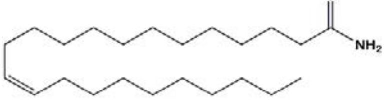
Additive	Chemical structure	Chemical formula	Molecular weight (g/mol)	Boiling point (°C)
Plasticizer:				
DEHP		$C_{24}H_{38}O_4$	390	384
DINP		$C_{26}H_{42}O_4$	418	403
DIDP		$C_{28}H_{46}O_4$	446	425
DEHA		$C_{22}H_{42}O_4$	370	417
PAs		$C_{33}H_{60}O_8$	584	
DBS		$C_{18}H_{34}O_4$	314	344
ATBC		$C_{20}H_{34}O_8$	402	327
acPG		$C_{19}H_{34}O_6$	358	287

Table 2.1 The chemicals properties of additives used in PVC gasket (continue)

Additive	**Chemical structure**	**Chemical formula**	**Molecular weight (g/mol)**	**Boiling point (°C)**
Plasticizer:				
DINCH		$C_{28}H_{52}O_4$	424	400
TAC		$C_9H_{14}O_6$	218	259
2-EHA		$C_8H_{16}O_2$	144	228
Stabilizer:				
ESBO		$C_{57}H_{98}O_{12}$	975	445
Slipping Agent:				
OA		$C_{18}H_{35}NO$	281	433
EA		$C_2H_{43}NO$	337	474

2.2.2 Derivatisation of Additive

A majority of plasticizers such as phthalates, adipates, sebacates, citrate and amides easily detect by GC, except ESBO was a large molecule of triglycerides and PAs was mixture of wide range of adipic ester compounds. ESBO and PAs were derivatized to smaller molecule of ester through transesterification under basic condition.

Transesterification is the process of exchanging the organic group of an ester (R_1) with the organic group of alcohol under basic condition. An alcohol need to be dry due to the water and humidity in the process promoted the saponification reaction and salts of fatty acid was produced which not desired.

Figure 2.5 showed the transesterification reaction of nucleophilic acyl substitution under basic condition. The strong base capable of deprotonating the alcohol makes a stronger nucleophile. The hydroxide nucleophiles attack at the eletrophilic C of ester group (C=O), breaking the π bond and creating the tetrahedral intermediate which has a negative charge on carbonyl oxygen. These electrons then fall back to the carbon and push off the alkyl forming the esters. This reaction makes a non-reversible mechanism.

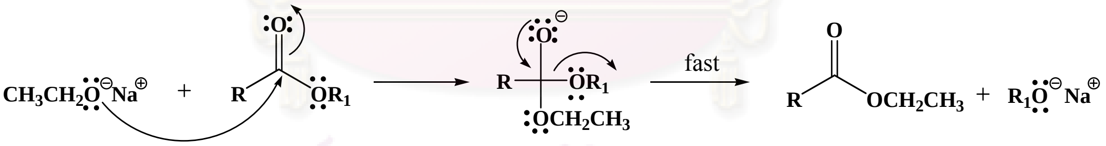


Figure 2.5 The mechanism of transesterification with ethoxide to form ethyl ester.

2.3 Theory of Migration

The transfer of chemicals from food packaging into its contents is known as migration. Migration is a diffusion process controlled by both kinetics (diffusion through the polymer and foodstuff) and thermodynamics (equilibrium partitioning between packaging and food) as described below:

2.3.1 Diffusion Theory

Diffusion process derives from a mass transfer, namely the Brownian motion of an individual migrant molecule from a high concentration region to a region of lower concentration. The diffusion process is a function of time, temperature, thickness of material, amount of chemical in the material, partition coefficient, and diffusion coefficient. It can be described by the diffusion mathematics derived from Fick's law [28-29]. Figure 2.6 shows the chemical diffusion in a polymer-solution interface.

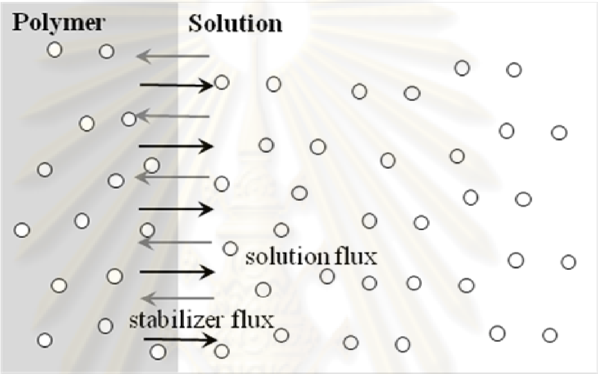


Figure 2.6 The diffusion in a polymer-food solution interface.

The mass transport by diffusion occurs throughout the storage of packed products; the Fick's 1st law is the simplest diffusion equation describing the mass transport in the polymer, whereas the Fick's 2nd law can be applied when the diffusion coefficient is a variable.

Fick's 1st law: $$F = -D_P\left(\frac{\delta C_P}{\delta x}\right) \quad (1)$$

Fick's 2st law: $$\frac{\delta C_P}{dt} = D_P\left(\frac{\delta^2 C_P}{\delta x^2}\right) \quad (2)$$

where F is the rate of transport per unit area of polymer; D is the diffusion coefficient of the migrant in the polymer (cm^2/s); C_P is the migrant concentration in the polymer (mg/g); x is the space coordinate measured to the polymer-food interface; t is the elapsed time (s)

2.3.2 The Diffusion Coefficient

The total amount of the migrant in the polymer to migrate into a fatty food or fatty food simulant at equilibrium, $m_{F,t}$ the most variables that control the migration of a substance are the contact time, t and temperature, T. The migration of a substance from a polymer into foods or food simulant occurs if $m_{F,t} / m_{F,e} \leq 0.5$:

$$\frac{m_{F,t}}{A} = 2kC_{P,0}\sqrt{\frac{D_P t}{\pi}} \tag{3}$$

where $m_{F,t}$ is the total amount of a substance migrated into food or food simulant over time, t; A is the contact surface area; t is contact time; D_P is the actual diffusion coefficient of the migrant in the polymer; $C_{P,0}$ is the initial concentration of the migrant in the polymer; k is a constant, which measures the influence of factors outside the polymer/migrant system and has a value of k = 1 by definition. In the absence of such influences and as long as no polymer is swelling k = 1.

The mass transport in opposite direction implies the migration of a substance from the foods or food simulant into polymer.

$$\frac{m_{P,t}}{A} = 2kC_{F,0}K\sqrt{\frac{D_P t}{\pi}} \tag{4}$$

where $m_{P,t}$ is the total amount of a substance migrated into polymer from food or food simulant over time, t; $C_{F,0}$ is the initial concentration of the migrating component in the simulant.

The influence of the simulant on the migration, the average migration distance made by the diffusing additive i in the polymer over time is defined x_i and its diffusion coefficient as $D_{P,i}$. Similarly, x_j is described as the distance, which the simulant migrated towards the polymer over time and $D_{P,j}$ is the diffusion coefficient of the simulant in the polymer. From the theory of diffusion the following ratio for x_i/x_j can be derived:

$$\frac{x_i}{x_j} = \sqrt{\frac{D_{P,i}}{D_{P,j}}} \tag{5}$$

In case of $x_i << x_j$, the polymer additive will be overrun by the simulant before it can migrate out of the polymer. If $x_i >> x_j$, the migration of polymer additive is not influenced by the migration of simulant.

2.3.3 The Partition Coefficient

The partition coefficient K of a substance between two phases depends on polarity of the substance and the polarity of the media; applying the rule "like dissolves like". For example, if filling a fatty product into a hydrophobic polymer, a complete partitioning of the fatty product from the polymer into foodstuffs takes place. The partition coefficient is defined as:

$$K_{P,F} = \frac{C_{P,e}}{C_{F,e}} \tag{6}$$

where $K_{P,F}$ is the partitioning coefficient of a substance between a polymer and foodstuff; $C_{P,e}$ and $C_{F,e}$ are the concentrations of substance in the polymer and food or food simulant at equilibrium, respectively.

An important quantity, which can be calculated at equilibrium, is the amount of a substance migrated into food or food simulant at equilibrium, $m_{F,e}$. This provides the migration potential in the polymer, i.e. the initial amount if the migrant is dissolved in the polymer, $m_{P,0}$. It can be derived from a mass balance calculated as follows:

$$m_{F,e} = \frac{m_{P,0}}{1 + K_{P,F}\frac{V_P}{V_F}} = \frac{m_{P,0}}{1 + K_{P,F}\,A\,d_P\frac{V_P}{V_F}} \tag{7}$$

where the volume of the polymer material of the packaging and the food or food simulant are V_P and V_F, respectively; A is the contact surface area; d_P is the layer thickness of the polymer.

In general, if $V_P/V_F << 1$ and at equilibrium $K_{P,F} \leq 1$; as an example in food containing fat, the total migration potential can be found in food simulant, so that $m_{F,e} = m_{F,0}$.

However, if the potential migrant is more soluble in the polymer than in the food simulant, $K_{P,F} >> 1$, at equilibrium only a small fraction can be found in the food simulant $m_{F,e} << m_{F,0}$. In that case, migration into aqueous food simulants occurs [21].

2.3.4 Migration Test

The aim of the migration testing is to ensure food safety and to protect the consumers by controlling the level of undesirable constituents entering the foodstuffs from the packaging [21]. Migration testing used for the investigation of ingredients such as monomers and additives used, as well as the amount of these substances present and their potential to migrate into food. Conventionally, the testing is performed as worst case in terms of temperature and contact between the food contact material and the food simulant. To provide comparable and consistent results of the migration test, four types of food have been legislated as food simulants by the EU as described in Directive 85/572/EEC:

Simulant A: distilled water, representing aqueous foodstuffs.
Simulant B: 3% acetic acid, representing acidic foodstuffs.
Simulant C: 10% ethanol, representing alcoholic foodstuffs.
Simulant D: rectified olive oil, isooctane or 95% ethanol, representing fatty foodstuffs.

The time and temperature conditions for the migration testing must be chosen as regulated for plastics in Directive 82/711/EEC [*28*]. For long-term storage at ambient temperature, simulation of exposure at 40 °C during 10 days must be performed. Additionally according to EN 13130-1 2004, as basic rule for migration testing, the jar is positioned on its head with the oil lying on the lids all the time to ensure the oil to be in direct and permanent contact with the gasket, [15].

2.4 Fundamentals of Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR FT-IR)

Attenuated total reflection Fourier transform infrared spectroscopy is one of the most employed surface characterization techniques using infrared radiation. The ATR is derived from the rapid decay of the evanescent field generated under the total internal reflection (TIR) phenomenon. As an electromagnetic radiation is traveling within an internal reflection element (IRE), a high refractive index material impinges the surface of the material with a low refractive index at an angle of incidence greater than the critical angle by a strong electromagnetic field, the so-called evanescent field, which is generated at the IRE/sample interface. The evanescent field is strongest at the interface with an exponential decay as a function of distance from the interface. If the sample is absorbing at the coupled frequency, the intensity of the reflected radiation is attenuated and becomes smaller than that of the incident radiation. The rapid decay of the evanescent makes an ATR spectrum very sensitive to physicochemical phenomenon at the surface of the sample [30 – 31].

2.4.1 A Principle of Light Reflection and Refraction

Refraction and reflection occur when the electromagnetic radiation travels from one medium into another with a different refractive index. The radiation passes through an incident medium with a refractive index n_1 into a medium with a refractive index n_2, a change of beam direction occurs (Figure 2.7). Refraction is described in Snell's law given by Equation 8 [32]:

$$n_1 \sin\alpha_1 = n_2 \sin\alpha_2 \tag{8}$$

where α_1 and α_2 are the angles of incident and refraction, respectively.

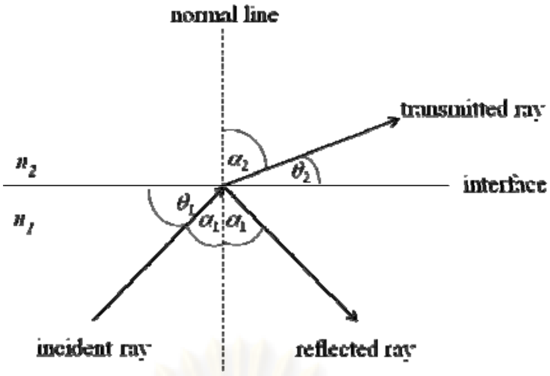


Figure 2.7 Reflection and refraction of a plane wave at a dielectric interface based on Snell’s law.

If $n_1 > n_2$, the light will reflect away from the normal line if the reflected angle α_2 equals 90°. The incidence angle α_1 is called the critical angle θ_C (Figure 2.8). The critical angle can be derived from Snell’s law given by Equation (9)

$$\theta_C = \sin^{-1}(n_2 / n_1) \tag{9}$$

If the incidence angle α_1 is greater than θ_C, the light will be totally internal reflected (TIR) at the interface.

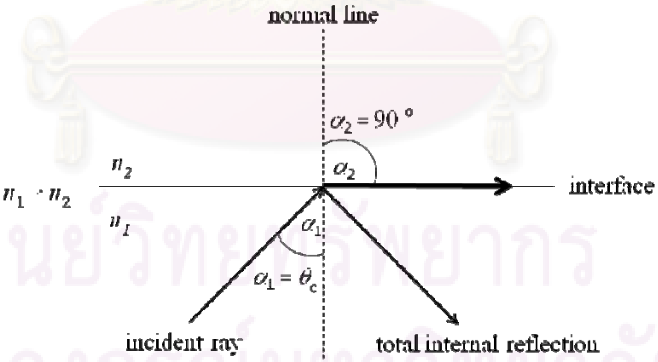


Figure 2.8 The light travels from an optical denser medium and impinges at the surface of the optically rare medium ($n_1 > n_2$) with an angle of incidence equal the critical angle. Total internal reflection occurs at an angle of incident greater than the critical angle.

When the rarer medium is absorbing, there is a reflection loss due to absorption. Reflectance of the beam leaving the interface is less than unity. This phenomenon is defined as the ATR phenomenon.

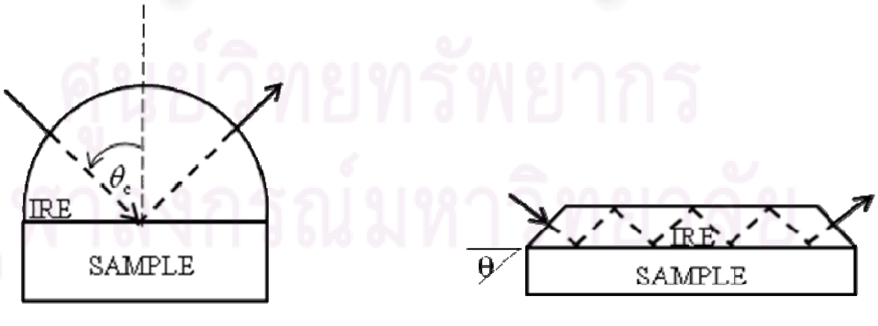
2.4.2 Internal Reflection Element (IRE)

The IRE is infrared transparent with a high refractive index. The optical properties of some infrared transmitting materials are displayed in Table 2.2 [33].

Table 2.2 Materials used for internal reflection elements.

Material	Usable wave number range (cm^{-1})	Reflective index at 1,000 cm^{-1}	Hardness (kg mm^{-2})
Silicon	8,300 – 1,500, 360 – 120	3.42	1,150
Germanium	5,500 – 600	4.02	780
Zinc selenide	20,000 – 460	2.43	120
Diamond	45,000 - ~2,500, ~1,600 – 200	2.417	8,820

The internal reflection crystal has many shapes, which can be divided into two main types; a single reflection type with a hemispherical IRE and a multiple reflection type with a trapezoidal as shown in Figure 2.9. The hemispherical IREs are used in variable-angle or fixed angle, single-reflection ATR attachments. The hemisphere allows the angle of incidence to be changed over a wide range without changing the divergence of the incident radiation. A choice must be made in the working angle or the range of angles of incidence, number of reflections, aperture, number of passes, surface preparation, and material from which it is made [34].



(A) Single reflection (B) Multiple reflection

Figure 2.9 IRE configuration commonly used in ATR experimental setups: (A) single reflection vibration-angle hemispherical crystal, (B) multiple reflection single-pass crystal.

This internal reflectance creates an evanescent wave that extends beyond the IRE/sample interface. The evanescent field is strongest at the surface of the sample and exponentially decays towards zero within a few microns from the surface of the sample [34 – 35].

2.4.3 Limitation of ATR FT-IR Spectroscopy

ATR FT-IR spectroscopy is a powerful technique for surface characterization based on molecular information. However, conventional ATR technique has several limitations such as the contact between IRE and the sample, the rough surface of sample leads to an air gap and if the air gap is thick enough, a correct spectrum of the sample cannot be observed [36]. Another limitation is in case of a large sampling area, a spectrum of a smaller sample cannot be obtained [37].

2.4.4 ATR FT-IR Microscopy

In ATR FT-IR microscopy an optical microscope is coupled to the infrared spectrometer. The microscope provides the morphology of the samples, whereas the IR is able to characterize the molecular bonding of the sample. This has facilitated the microscopy of samples to detect trace contamination on the surface of the sample. The optical diagram of an infrared microscope is shown in Figure 2.10 [35, 38 – 39]. An infrared radiation tracing within the 15X Schwarzschild-Cassengrain infrared objectives, the focused radiation reflected onto the focusing plane with angle of incidence ranging 15.6°-35.5° are shown in Figure 2.11. The coupling-focused radiation into specially designed Ge μIRE and ATR phenomenon on the interface can be seen.

The designed hemispherical dome of the cone-shaped Ge-IRE facilitates the focused radiation to travel into the μIRE by minimizing the reflection loss at the air/Ge interface. Thus, a good contact with the sample is achieved, minimizing the force on the tip, eliminating interferences from internal reflection, and providing good sensitivity.

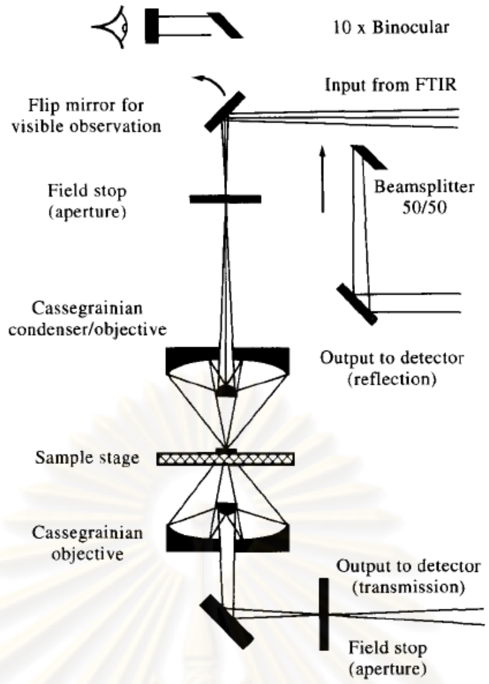


Figure 2.10 The optical diagram of the infrared microscope.

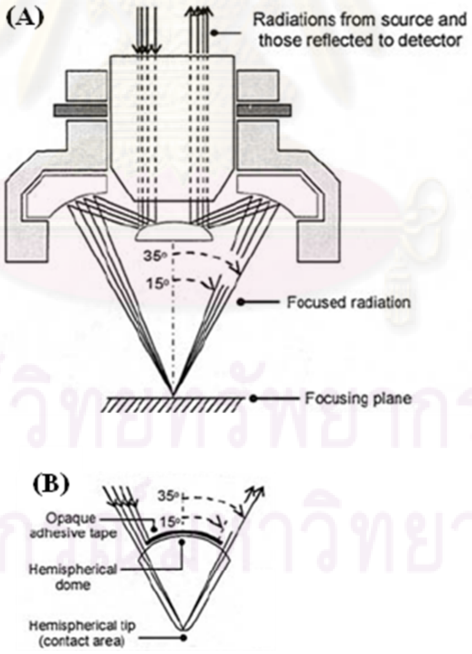


Figure 2.11 (A) Infrared radiation tracing within 15X Schwarzschild-Cassengrain objective of the infrared microscope. (B) Infrared tracing within homemade GE μIRE.

CHAPTER III
EXPERIMENTAL

3.1 Instruments and Apparatus

3.1.1 Gas Chromatography (GC): Module 7890A consists of auto-injector, auto-sampler and flame ionization detector. Agilent Technologies, U.S.A.

3.1.2 Gas Chromatography (GC): Module 7890A consists of auto-injector, auto-sampler and mass spectrometer module 5975C. Agilent Technologies, U.S.A.

3.1.3 Gas Chromatography (GC): Module Trace GC Ultra^{TM} consists of auto-injector, auto-sampler, PVC injector and mass spectrometer a module DQS^{TM} II. Thermo Scientific, U.S.A.

3.1.4 Fourier Transform-Infrared Spectroscopy (FT-IR): Module Nicolet 6700 consists of a mercury-cadmium-telluride (MCT) detector, Continuµm^{TM} infrared microscope with 15X Cassengrain infrared objective and 10X glass objective, variable-angle single-reflection attenuate total reflection (ATR) accessory (The Seagull^{TM}, Harrick Scientific. U.S.A.) with a hemispherical germanium (Ge) IRE (32 mm diameter) and homemade slide-on germanium (Ge) µATR accessory with cone shaped Ge as IRE. Thermo Electron Corporation, U.S.A.

3.1.5 A pre-column: DB-1, 0.5 m × 0.25 mm I.D. × 0.1 µm film thickness, Agilent Technologies, U.S.A.

3.1.6 Universal press-fit T-piece: fused silica tubing, 13 cm × 0.15 mm I.D., Agilent Technologies, U.S.A.

3.1.7 GC column: DB-5, 30 m × 0.25 mm I.D. × 0.25 µm film thickness, Agilent Technologies, U.S.A.

3.1.8 GC column: Rtx-2330, 15 m × 0.25 mm I.D. × 0.20 µm film thickness, Restek, U.S.A.

3.1.9 GC column: Rtx-225, 15 m × 0.25 mm I.D. × 0.25 µm film thickness, Restek, U.S.A.

3.1.10 Vortex mixture, Scientific Industries.

3.1.11 Beakers 10, 25, 250, 1000mL.

3.1.12 Volumetric flask 10.00, 25.00, 50.00 mL.
3.1.13 Graduated cylinders 10.0, 50.0 mL.
3.1.14 Erlenmeyer flasks 250.0 mL.
3.1.15 Volumetric pipette 5.00 mL.
3.1.16 Micro-pipettes and tips.
3.1.17 GC vials 1.5 mL with caps, Agilent Technologies, U.S.A.
3.1.18 Screw cap amber vials 24 mL with PTFE septum.
3.1.19 Screw cap vials 50 mL with PTFE septum.
3.1.20 Micro-syringe 10 μL, Hamilton.
3.1.21 Incubator
3.1.22 GC oven: a module 5890A, Agilent Technologies, U.S.A.
3.1.23 Lint-free paper
3.1.24 Glass slide
3.1.25 Stirring rod
3.1.26 Nujol

All glass apparatus was washed thoroughly in detergent, rinsed with double distilled water and then rinsed with suitable organic solvent before used.

3.2 Chemicals

3.2.1 The Standard Compounds

1-Ethylnaphthalene (**EN**), Erucamide (**EA**), Di-(2-ethylhexyl) phthalate (**DEHP**) and Diisononyl phthalate (**DINP**) (Aldrich, Germany). Cyclohexyl acetic acid (**CHAA**), Dimethyl pimelate (**DMP**), Di-(2-ethylhexyl) adipate (**DEHA**) and *tri*-acetyl butyl citrate (**ATBC**) (Aldrich, U.S.A.). Diheptyl phthalate (**DHP**) (Aldrich, United Kingdom). Epoxidized soybean oil (**ESBO**) and Diepoxy methyl eicosanoate (**C20:2**) (Fluka, Germany). Diisodecyl phthalate (**DIDP**) (Fluka, Japan). Dibutyl sebacate (**DBS**) (Sigma, Japan). Oleamide (**OA**) (Sigma, U.S.A.). Acetylated Partial Glyceride (**acPG**), Diisononyl-cyclohexane-1,2-dicarboxylate (**DINCH**), Dipropyl sebacate (**DPS**), Polyadipates (**PAs**) and Triacetin (**TAC**) were supported by Official Food Control Authority of the Canton of Zürich (Kantonales Labor Zürich), Zürich, Switzerland.

3.2.2 Organic Solvents

Heptane and tetrahydrofuran (Fluka, Germany), 1,4-dioxane (Carlo-Erba, Germany), *tert*-butyl methyl ether, hexane (Merck, Germany) were of analytical grade.

3.2.3 Reagents

Disodium hydrogen citrate sesquihydrate (Aldrich, Germany), sodium (Aldrich, France), ethanol, methanol and acetone (Merck, Germany) were of analytical grade.

3.2.4 Olive oil used as fatty food simulant according to EU regulations.

3.3 Metal Lid Samples

Two types of the lug caps were chosen for the study; each type was received from the same batch of products and the same lid manufacturer. Both caps were used for the study of the gasket composition and the study of the parameters that affect to migration of plasticizers into fatty food simulant.

Parameter	Type I	Type II
Lug cap		
Diameter (mm)	48	63
Weight (g)	6.6	11.0
Width of gasket inside of the lid(mm)	13	13
Weight of gasket (g)	0.99	1.23
Glass jar		
Diameter (mm)	51.2	67.2
Brimful (mL)	115.0	228.9
Height (mm)	86.5	96.0
Weight (g)	105	156

3.4 Determination of Gasket Composition

3.4.1 The Study of ATR FT – IR Microspectroscopy

ATR FT-IR microspectroscopy was employed for surface characterization of the gasket for identification of plasticizers. This technique possesses several advantages such as little sample preparation, no chemicals used, non-destructive sample, fast and easy to operate. Three types of collecting techniques were studied for investigating plasticizers in the gasket; normal mode, contact-and-collect mode and absorbance enhancement. All ATR spectral were acquired with a homemade slide-on Ge µATR accessory coupled with a Continuµm^{TM} infrared microscope connected to a FT-IR spectrometer.

3.4.1.1 Homemade Slide-on Ge µATR Accessory

The homemade slide-on Ge µATR accessory was developed from a commercial ATR accessory with hemispherical IRE which was limited from a large sampling area (5×5 mm^2), limited by size of sample holder which resulted in a poor contact between the sample and IRE. The homemade slide-on Ge-µATR accessory consisted of two components; (1) the slide-on housing which was designed for placing the slide-on Ge-µIRE on the objective infrared microscope, (2) the slide-on Ge µIRE which was designed for mounting the cone-shaped Ge IRE and slide into the slide-on housing. The homemade slide-on Ge-µATR accessory was composed on the 15X Schawarazschild-Cassegrain infrared objective which connected to the Nicolet 6700 FT-IR spectrometer as showed in APPENDIX B. The incident radiation from the infrared microscope was coupled into the dome-shaped Ge IRE and underwent total internal reflection at the tip of Ge, the amount of light was controlled by aperture of the microscopy. Spectral acquisitions were performed in the reflection mode.

3.4.1.2 Spectral Acquisition Parameter

Nicolet 6700 FT - IR Spectrometer:	
Instrumental setup	
Source	Standard Globar ™ Infrared Light Source
Detector	Mercury-Cadmium-Telluride (MCT)
Beam splitter	Ge-coated KBr
Acquisition Parameters	
Spectral resolution (cm^{-1})	4
Number of scans	64 scans
Mid-infrared range (cm^{-1})	4000 – 650
Continuμm ™ Infrared microscope:	
Instrumental setup	
Detector	Mercury-Cadmium-Telluride (MCT)
Objective	15X Schawarazschild-Cassegrain
Aperture size	150 × 150 μm

3.4.1.3 Experimental Procedure

A piece of gasket sample was cut to 1× 2 cm^2 and the underlying coating from the inner part of the lug cap was removed.

3.4.1.3.1 Normal Mode: The sampling technique feature in the tip of Ge μIRE directly contacted with the gasket surface. The obtained spectra associated with the molecular structure of component that the tip of Ge μIRE throughout contact on the gasket.

3.4.1.3.1.1 The slide-on Ge μIRE was inserted onto the slide-on Ge housing and the tip of Ge μIRE was cleaned with lint-free paper soaked with acetone.

3.4.1.3.1.2 A piece of gasket was placed on the microscope stage and the slide-on Ge μIRE was placed into a pre-aligned position above a sample.

3.4.1.3.1.3 The background spectrum was collected through the Ge μIRE when it was not in contact with a sample.

3.4.1.3.1.4 The microscope stage was elevated until the selecting area contacted with the tip of Ge μIRE and stopped when the light of LED alert sensor was lit.

3.4.1.3.1.5 The ATR spectra was collected via Ge μIRE which contacted with a sample.

3.4.1.3.1.6 The microscope stage was lifted down and the sample was removed.

3.4.1.3.1.7 The step 3.4.1.3.1.4 – 3.4.1.3.1.6 was repeated 5 times.

3.4.1.3.2 Contact-Collect Mode: The sampling technique was used for measuring the molecular structure of constituent species on the gasket sample that stuck at the tip of Ge μIRE. The acquired spectra merely showed the composition at the surface of the gasket.

3.4.1.3.2.1 The steps 3.4.1.3.1.1 – 3.4.1.3.1.4 were performed, respectively.

3.4.1.3.2.2 The microscope stage was lifted down and sliding the microscope stage to another position on the gasket.

3.4.1.3.2.3 The microscope stage was elevated until the tip of Ge μIRE contacted the new selected area and stopped when the light of LED alert sensor was lit.

3.4.1.3.2.4 The step 3.4.1.3.2.2 – 3.4.1.3.2.3 was repeated 5 times; the contaminants from the surface of the gasket were stuck and accumulated on the tip of Ge μIRE by repeatedly making contact between the tip and the surface of a sample.

3.4.1.3.2.5 The ATR spectra was collected when the Ge μIRE did not make contact with the sample.

3.4.1.3.3 Absorbance Enhancement: The sampling technique used for enhancing the signal of ATR spectra of contact-and-collect mode by using mineral oil as an enhancing agent that soaked on the tip of Ge μIRE. The higher spectra of the surface component related with the high amount at the tip of Ge μIRE.

3.4.1.3.3.1 The slide-on Ge μIRE was inserted onto the slide-on Ge housing and the tip of Ge μIRE was cleaned with lint-free paper soaked with acetone.

3.4.1.3.3.2 The background spectrum was collected through the Ge μIRE when it was not in contact with a sample and then the slide-on Ge μIRE was removed.

3.4.1.3.3.3 The mineral oil was dropped on the glass slide and smeared to a thin film with the stirring rod.

3.4.1.3.3.4 The tip of Ge μIRE was touched on the thin film and inserted onto the slide-on Ge housing.

3.4.1.3.3.5 The ATR spectrum was collected same as step 3.4.1.3.2.

3.4.2 The Study of Gas Chromatography

Gas chromatography is an analytical method for determining the plasticizers for a long time. A majority of plasticizers as the ester compounds are easily analyzed by GC, except ESBO and PAs must be transesterification to smaller ester compounds render them enable to GC-FID as referred Biedermann-Brem et al [5].

In this study, all 14 of standard plasticizers were divided to three groups as followed in Table 3.1, in order to easily identify and for cover all plasticizers in the gasket. All plasticizers were prepared via direct analysis and transesterification in the same extracted solution of gasket.

Table 3.1 The group of standard plasticizers for gasket composition study.

	Direct analysis	Transesterification
SET I	2-EHA, OA, DEHP, EA and DIDP	ESBO
SET II	TAC, DBS, ATBC, DEHA, DINCH	-
SET III	acPG, DINP	PAs

3.4.2.1 Preparation of Standard Solution

3.4.2.1.1 Stock Internal Standard Solution in Tetrahydrofuran

The stock internal standard solutions for 1,000.0 ppm of each EN and DMP were prepared by dissolving 0.2500 g of EN and DMP, then diluting them to the mark with tetrahydrofuran in 250 mL volumetric flask.

3.4.2.1.2 Standard Mixture Solutions

Set I: The 5,000.0 ppm of each ESBO, DIDP and 1,000.0 ppm of each OA, EA, DEHP were prepared by weighing 0.0500 g of each ESBO, DIDP and weighing 0.0100 g of each OA, EA, DEHP and dissolving them to the mark with solution in 3.4.2.1.1 in 10 mL volumetric flask.

Set II: The 5,000.0 ppm of DINCH and 1,000.0 ppm of each DEHA, ATBC, DBS, TAC were prepared by weighing 0.0500 g of each DINCH and weighing 0.0100 g of each DEHA, ATBC, DBS, TAC and dissolving them to the mark with solution in 3.4.2.1.1 in 10 mL volumetric flask.

Set III: The 5,000.0 ppm of DINP and 1,000.0 ppm of each acPG, PAs were prepared by weighing 0.0500 g of each DINP and weighting 0.0100 g of each acPG, PAs and dissolving them to the mark with solution in 3.4.2.1.1 in a 10 mL volumetric flask.

The standard solutions for preparation of the response factor were prepared from these solutions.

3.4.2.2 The Study of Gas Chromatography Conditions

The GC condition was developed by varying the oven temperature in order to obtain the optimum GC conditions. Then injection of 1 μL of internal standard solution and standard solutions and sample solutions to the GC optimum condition were evaluated.

3.4.2.3 The Study of Transesterification Kinetic

ESBO and PAs were derivatized to smaller molecule of ethyl ester through transesterification under basic condition of sodium ethoxide prior to detectable by GC. The transesterification is a fast reaction as well as become faster saponification which not desired. This study finds out suitable reaction time for transesterification and stopped before saponification appeared.

3.4.2.3.1 The 2.50 mL of internal solution in 3.4.2.1.1 was pipette and added 5.00 mL of ethanol into 10 mL amber vial.

3.4.2.3.2 The 1.00 mL of these solution and 0.65 mL of 15% ethoxide were mixed into 10 mL amber vial, and left to react 1 to 10 minutes.

3.4.2.3.3 The 4.00 mL of 60% *tert*-butyl methyl ether in hexane and 4.00 mL of 10% disodium hydrogen citrate were added immediately to the solution to stop the reaction, and then shaken vigorously.

3.4.2.3.4 The phase was separated and takes the upper phase to analyze by GC.

3.4.2.3.5 The 5,000 ppm of ESBO and 1,000 ppm of PAs, DEHA were carried out followed 3.4.2.3.1 – 3.4.2.3.4, respectively.

3.4.2.4 The Study of Response Factor

The procedure to study the response factor of standard plasticizers can be described as follows:

3.4.2.4.1 The concentrations of standard solutions were 500.0 and 2,500.0 ppm. The 2.50 mL of each standard mixture solutions from 3.4.2.1.2 and 5.00 mL of ethanol were mixed into 10 mL of amber vial.

3.4.2.4.2 The direct analysis was prepared by pipetting 0.25 mL of each standard mixture solution from 3.4.2.4.1 were added into 2 mL of autosampler vial and diluting them with 0.75 mL of *tert*-butyl methyl ether and analysed by GC.

3.4.2.4.3 The transesterification was prepared by pipetting 1.00 mL of each standard mixture solution from 3.4.2.4.1 and 0.65 mL of 15% ethoxide was mixed into 10 mL of amber vial, and left to react following the result from 3.4.2.3. Then 4.00 mL of 60% *tert*-butyl methyl ether in hexane and 4.00 mL of 10% disodium hydrogen citrate were added immediately to the solution to stop the reaction. It was then vigorously shaken and takes an upper phase of 60% *tert*-butyl methyl ether in hexane to analyze by GC.

3.4.2.5 The Procedure for Extraction

The extraction procedure of plasticizers from the gasket can be described as followed:

3.4.2.5.1 The 50.00 mg of gasket material without food contact was dissolved in 2.50 mL of tetrahydrofuran contained internal standard from 3.4.2.1.1, which usually required 15-30 minutes for the gasket to dissolve completely. A turbid solution was precipitated by admixture 5.00 mL of ethanol.

3.4.2.5.2 A supernatant was extracted similarly with the direct analysis and the transesterification from the step 3.4.2.4.2 and 3.4.2.4.3.

3.4.2.5.3 The lug cap 48 and lug cap 63 mm were analyzed for 6-replications and followed by step 3.4.2.5.1 – 3.4.2.5.2, respectively.

3.5 The Migration Testing into Fatty Food Simulant

The migration testing by simulation used for studied the plasticizers potentially migration from gasket into fatty food simulant under the studied conditions. Olive oil was used as instead of real food in order to decrease or get rid of the problem from interferences in real foods. The simulation method referred the standard method of EU [1 – 4, 15].

3.5.1 Procedure of The Simulation for Migration Testing

The conditions for migration test into fatty foods simulant (olive oil) such as contact time, contact temperature, initial hating (pasteurization) and size of surface contact area were obtained from these simulations, which can be described as follows:

3.5.1.1 The jar was closed according to the recommendations of the producer. The lug was closed between 5 – 10 mm from the seam of the jar neck. Then the jar was heated in the incubator to 100 °C for 15 minutes in order to render the gasket soft and deform it as in an industrial application.

3.5.1.2 10.00 g of olive oil was poured into the jar and then closed tightly.

3.5.1.3 The jar was turned over. For the study of contact time, the inverted jar was kept in GC oven at 30 °C for 30 days.

3.5.1.4 The jar was turned on its lid. For the study of pasteurization, the inverted jar was heated in GC oven to 100 °C for 1hour, then cooled and kept in GC oven at 30 °C for 30 days.

3.5.1.5 The jar was turned on its lid. For the study of contact temperature, the inverted jar was heated in GC oven to 30 °C, 40 °C and 60 °C for 10 days.

3.5.1.6 The study for size of surface contact area was simulated in the same way with the step 3.5.1.3 – 3.5.1.5 for lug cap 48 mm and lug cap 63 mm.

3.5.1.7 At the end of time required for the simulated condition, the jar was shaken vigorously 10 times and the oil poured out and analysed.

3.5.2 The Study of ESBO in The Fatty Food Simulant

The method extraction of ESBO from fatty food simulant without extraction of the lipid was used in this work. The large molecule of ESBO in fatty food simulant was transesterified to smaller methyl esters included 26% of epoxy oleate, 54% of diepoxy linoleate and 11% of triepoxy linolenate. The two of diastereomers of methyl diepoxy linoleate was required as quantification analyte of ESBO, this work was referred Fankhauser-Noti et al [13].

3.5.2.1 Preparation of Standard Solutions

3.5.2.1.1 Stock Standard Solutions in 1,4-Dioxane

The 1,000 ppm stock standard solutions of each ESBO and internal standard (C20:2) were prepared by dissolving 0.0100 g of each standard and diluting them to the mark with 1,4-dioxane 10.00 mL volumetric flask.

3.5.2.2 The Study of Gas Chromatography- Mass Spectrometry Conditions

The GC condition was developed by varying the oven temperature in order to obtain the optimum GC conditions. The 1 μL of standard solutions and sample solutions were injected under GC optimum condition.

3.5.2.3 The Study of Response Factor

The procedure to study the response factor of ESBO can be described as follows:

3.5.2.3.1 The concentration of standard solutions was 10 ppm. The 100 μL of each stock standard solution and 4.00 mL of 1, 4-dioxane was mixed into 50 mL of vial.

3.5.2.3.2 Transesterification occurred by adding 5.00 mL of 5% methoxide into the mixture, and allowing react for 1 minute. Then 4.00 mL of 15% disodium hydrogen citrate and 4.00 mL of heptanes were added and shaken intensively.

3.5.2.3.3 The phase separated clearly, takes an upper phase of heptane to analyze.

3.5.2.4 The Procedure for Extraction

The extraction procedure of ESBO in the olive oil can be described as follows:

3.5.2.4.1 A portion of 100.0 mg of fatty food simulant from step 3.5.1.7 was spiked with 6 μL of internal standard solution, then dissolving the mixture solution with 4.00 mL of 1,4-dioxane in 50 mL vial.

3.5.2.4.2 The extracted solution was transesterified in the same way with the step from 3.5.2.3.2 – 3.5.2.3.3, respectively.

3.5.3 The Study of Monomeric Plasticizers in The Fatty Food Simulant

The method for determination of monomeric plasticizers in fatty food simulant in this work was separation of fatty matrix occurred during injection by using programmed temperature vaporizing injection GC-MS as referred Fankhauser-Noti et al [20].

3.5.3.1 Preparation of Standard Solutions

3.5.3.1.1 Stock Standard Solution in *tert*-Butyl methyl ether

The 10,000 ppm stock standard solutions of each compound were prepared by dissolving 0.1000 g of each standard and diluting them to the mark with *tert*-butyl methyl ether in 10 mL volumetric flasks.

3.5.3.1.2 Internal Standard Mixture Solution

The 1,000 ppm of each internal standard solution was prepared by pipetting 1,000 µL of DHP and 33 µL of 30% DPS, then diluting them to the mark with hexane in 10 mL volumetric flask.

3.5.3.1.3 Standard Mixture Solution

The 100 ppm of standard mixture solutions were prepared by pipetting 100 µL of each 10,000 ppm stock solutions, then diluting them to the mark with hexane in 10 mL volumetric flask.

3.5.3.2 The Study of Gas Chromatography- Mass Spectrometry Conditions

The GC condition was developed by varying the oven temperature in order to obtain the optimum GC conditions. The 1 µL of standard solutions and sample solutions were injected to the GC under optimum conditions.

3.5.3.3 The Study of Standard Calibration Curves

The procedure to study the calibration curves of standard TAC, DBS and DINCH can be described as follows:

3.5.3.3.1 200.0 mg of olive oil without plasticizers was diluted with 10.00 mL of 20% *tert*-butyl methyl ether in hexane, then spiking 20 µL of the internal standard mixture solution from step 3.5.3.1.2.

3.5.3.3.2 The concentration of standard solutions: 1.5, 3.0, 6.0, 12.5, 25.0, 100.0, 200.0 and 400.0 ppm were prepared by pipetting the standard mixture solutions from 3.5.3.1.3 and diluting them with 1.00 mL of the mixture solution from step 3.5.3.3.1.

3.5.3.3.3 The standard calibration solutions were injected from 1.5 ppm to 400.0 ppm, respectively into GC under the optimum condition.

3.5.3.3.4 The relationship between concentration and peak area were plotted. The intercepts, slopes and correlation coefficients from curve are evaluated.

3.5.3.4 The Procedure for Extraction

The extraction procedure of monomeric plasticizers in the olive oil can be described as follows:

3.5.3.4.1 A portion of 100.0 mg of fatty food simulant (olive oil) from 3.5.1.7 was spiked with 10 µL of the internal standard mixture solution, then extracting with 5.00 mL of 20% *tert*-butyl methyl ether in hexane.

3.5.3.4.2 The 100 µL of extracted solution was diluted with 900 µL of 20% *tert*-butyl methyl ether in hexane into 2 mL of autosampler vial and added 1 dropped of olive oil was added without plasticizers before analysis.

CHAPTER IV
RESULT AND DISCUSSION

4.1 The Result of Determination of Gasket Composition

4.1.1 ATR FT-IR microspectroscopy

In this research, the developed ATR FT-IR microspectroscopy with homemade slide-on Ge μATR accessory was employed for gasket characterization. The new gasket of lug cap 48 mm and 63 mm were identified by three modes of collection sample. The ATR FT-IR spectrums were shown in Figure 4.1.

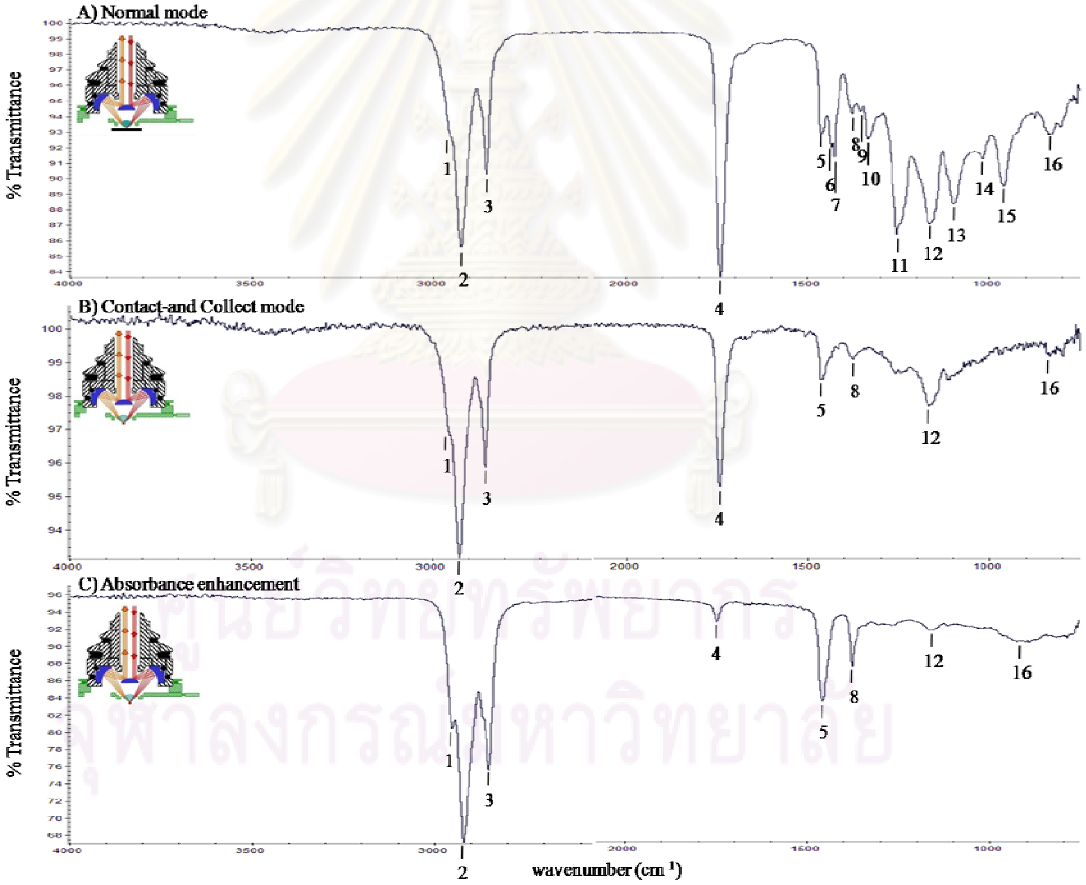


Figure 4.1 ATR spectra of new gasket acquired by three modes of sample collection; (A) Normal mode, (B) Contact-and-Collection mode and (C) Absorbance Enhancement.

The observed ATR spectra of all sampling techniques were not much difference. However, the relative absorbance of the normal mode was higher than the absorbance enhancement and contact-and-collect mode, respectively. This is because normal operation provided the better contact between the Ge μIRE/gasket, as well as the long contact time that make high spectra acquisition compared to the contact-and-collect operation and absorbance enhancement. So, the ATR spectra of normal operation showed components of plasticizer in the gasket and also polymer-based that the tip of Ge μIRE throughout contact. The contact-and-collect operation and absorbance enhancement were the technique features, the trace of components on the surface of gasket were able to be identified. When the tip of Ge μIRE contacted the components on the surface of gasket, the components were cumulatively collected on the tip of Ge μIRE. ATR spectra of component that stuck on the tip were obtained without the matrix interference from polymer-based. On the other hand, ATR spectra was improved by smearing an enhancing agent on the tip of Ge μIRE render dissolved and increasing the amount of components on the tip of Ge μIRE. Therefore, the ATR spectrum of absorbance enhancement was higher acquisition spectra than contact-and-collect mode.

The percent matching library show the degree of identical of sample spectra with reference spectrum. The reference spectra were referred from 2 library sources such as Hummel Polymer Sample Library which included 2,011 spectra of polymer and additive polymers as well as The User Sample Library which obtained from 14 spectra of the standard plasticizers were used in the PVC gasket. Table 4.1 showed the name of expected compounds including with the percent matching libraries of these three sampling techniques. The quantitative information from ATR FT-IR microspectroscopy of the new gasket samples confirmed that they contained with ESBO, TAC, DBS and DINCH.

Table 4.1 The percent matching library of new gasket compared to the standard plasticizers spectra

Collection mode	Percent matching library (%)				
	PVC	ESBO	TAC	DBS	DINCH
Normal	52.15	81.54	29.33	51.63	35.44
Contact-and-Collect	-	50.84	14.73	12.79	33.59
Absorbance enhancement	-	65.98	23.90	34.73	23.74

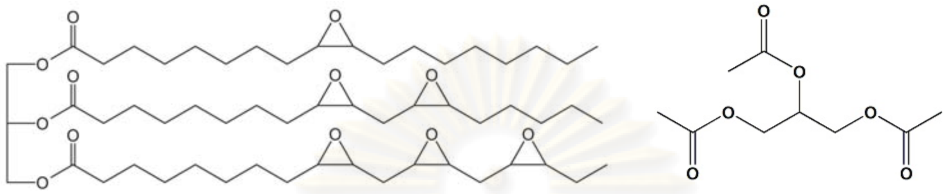
Although, ATR spectra of normal operation showed high percent matching with reference spectra of plasticizers but the spectra at fingerprint region (1,500-750 cm^{-1}) revealed a matrix interference of PVC with as a polymer-based of gasket as showed in APPENCIX C. The complication of this matrix interference spectrum was subtracted prior to identify the sample spectra that consist of various types in gasket sample. For contact-and-collect operation was able to elimination the matrix interference, which easier to identify the components in the gasket sample. Furthermore, the improvement of the intensive of sample spectrum was easily to carry out by absorbance enhancement technique as described above.

Table 4.2 The wavenumber of the functional groups of plasticizer component in the gasket

No.	Wavenumber (cm^{-1})			Functional group
	Normal	Contact-and collect	Absorbance enhancement	
1	2,952	2,952	2,952	C-H stretching of CH_3, CH_2 and CH in ESBO
2	2,923	2,925	2,921	
3	2,853	2,853	2,852	
4	1,739	1,743	1,747	C=O stretching of ester of ESBO and TAC
5	1,462	1,466	1,458	C-H bending of CH_2 ester of DBS
6	1,437	-	-	C-H bending of CH_3 of PVC
7	1,426	-	-	
8	1,377	1,377	1,376	C-H bending of CH_3 of ester of DINCH
9	1,354	-	-	C-H bending of CH_3 of PVC
10	1,333	-	-	
11	1,254	-	-	
12	1,164	1,166	1,162	C-O stretching of ester of DINCH
13	1,097	-	-	C-Cl of poly(vinyl chloride)
14	1,016	-	-	
15	958	-	-	
16	802	798	788	C-O-C of epoxy group in ESBO

The plasticizers were ester molecules that were composed of the functional group of esters and epoxy groups especially for ESBO, as showed in Figure 4.2 and Table 4.2 showed the wavenumber of the functional group of plasticizers composed in the new gasket samples.

(A) Epoxidized soybean oil (ESBO) (B) Triacetin (TAC)



(C) Diisononyl cyclyhexane-1,2-dicarboxylate (DINCH) (D) Dibutyl sebacate (DBS)

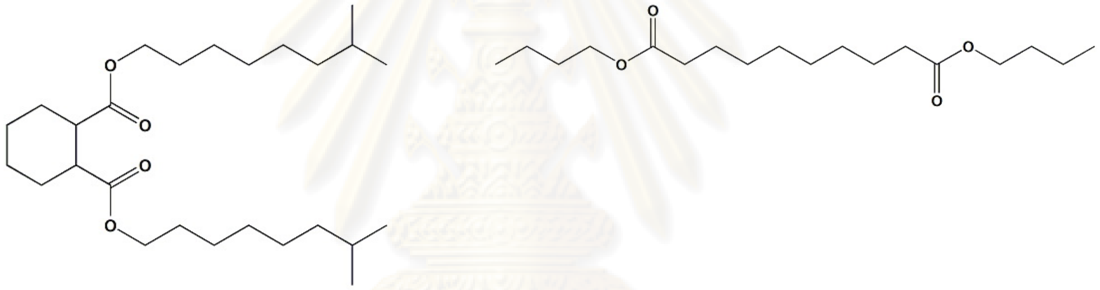


Figure 4.2 Compounds and structures of plasticizers formed in the gasket samples (A) ESBO, (B) TAC, (C) DINCH and (D) DBS.

ATR spectra of new gasket observed the characteristic band of plasticizers as followed by: the ESBO was observed at 2,923 and 2,854 cm^{-1} correspond with –CH vibration, the –CO stretching of ester at 1,743 cm^{-1} and the epoxy group of ESBO present at 829 cm^{-1}. The DBS present at 1,466 cm^{-1} of –CH_2 vibration. The wavenumber at 1,377 cm^{-1} indicated ester group of DINCH. TAC presents the ester group at 1,739 cm^{-1}. On the other hand, the several bands at the fingerprint region of normal operation corresponded with –CH bending of PVC and C–Cl revealed at the 958 cm^{-1} that are the polymer-based interference.

The result of determination of gasket composition by ATR FT-IR microspectroscopy of lug cap 48 mm and 63 mm showed the identical spectrum. The collection techniques of normal mode, contact-and-collect mode and absorbance enhancement were able to identify that plasticizers consist of ESBO, TAC, DBS and DINCH. However, ATR FT-IR microspectroscopy is a qualitative tool that applied for screening method of plasticizers in the gasket due to a fast and easy technique.

4.1.2 Gas Chromatography

The injection volume of 1 μL was used for all samples. The optimum condition was reported in Table 4.3. The chromatograms of standard mixture Set I – Set III were shown in Figure 4.4 – 4.6.

Table 4.3 Gas chromatography conditions for analysis of plasticizer in gasket.

GC Parameters	GC Conditions
Analytical column	DB-5, 30 m × 0.25 mm i.d. × 0.25 µm
Temperature program	Initial 60 °C for 1 min, 15 °C/min to 90 °C , 5 °C/min to 140 °C and 15 °C/min to 320 °C
Injection mode	Splitless mode
Injection temperature	270 °C
Carrier gas	Helium, 3mL/min
Detector	Flame Ionization Detector, FID

4.1.2.1 Transesterification Kinetic

The transesterification reaction was studied at room temperature during 1 – 10 minutes. The peak area ratio of standard plasticizers such as DMP_{IS} (as internal standard), ESBO, PAs and DEHA were calculated for checking the suitable reaction time for transesterification that in order to avoid saponification. The peak area ratio of plasticizers obtained from the peak area of transesterified standard plasticizers was divided by the peak area of inert internal standard (EN_{IS}) which no effect from transesterification reaction. Figure 4.3 showed the transesterification kinetic of standard plasticizers to be transesterified substance which related to reaction time.

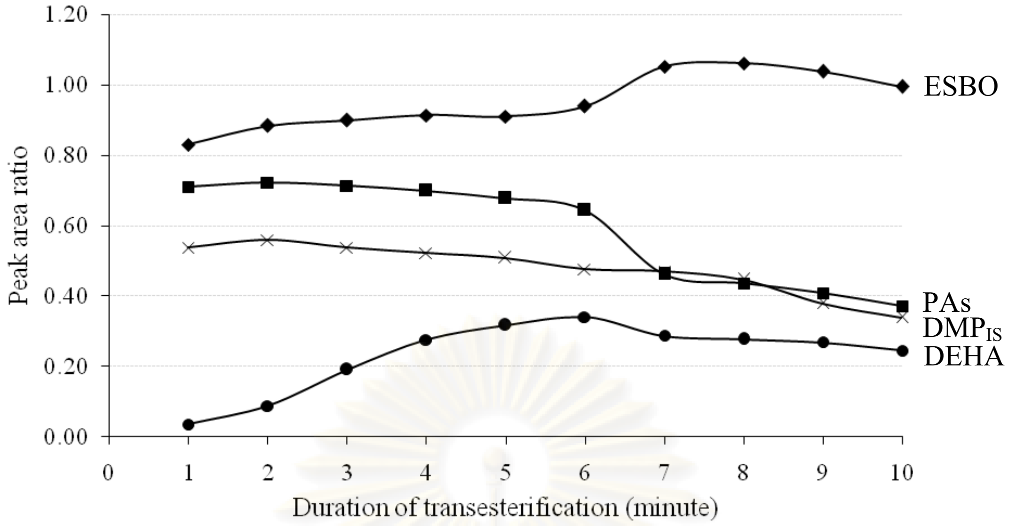


Figure 4.3 Peak area ratios indicate the reaction time of transesterification.

The decreasing of peak area ratio of plasticizers related to the increasing of fatty acid from saponification reaction appeared. The result showed, the peak area of ESBO was slightly increased, and after 10 minutes the peak area was decreasing that indicating the saponification appeared. For internal standard, PAs and DEHA, the peak areas were increasing, and after 6 minutes the peak areas were decreasing which the saponification was indicated. Therefore, the reaction time of transesterification for ESBO and PAs were indicated during 6 minute. This reaction time was applied for study of the response factor value of ESBO and PAs and analysis procedure of gasket composition.

Figure 4.4 – Figure 4.6 showed the chromatogram of the standard plasticizers that obtained by direct analysis and transesterification as followed in Table 3.1. However, both sample preparation steps were carried out at the same time, for identification and quantification of monomeric plasticizers used the chromatogram of direct analysis as well as ESBO and PAs used chromatogram of transesterification. Furthermore, transesterification enable to confirm constituents observed by direct analysis as described below.

Figure 4.4A, the chromatogram of direct analysis of standard mixture Set I showed the elution order of compounds as 2-EHA, OA, DEHP, EA and a hump of DIDP, respectively. For transesterification (Figure 4.4B) reveals the components not observed in direct analysis, ESBO consists of 5 main ethyl esters derivatives such as palmitic (C16:0), stearic (C18:0), ethyl epoxy oleate (C18:1E), ethyl diepoxy linoleate (C18:2E) and ethyl triepoxy linolenate (C18:3E). In addition, the C18:1E, C18:2E were mainly components in ESBO composition (about 80 %), for these reasons the sum of both compounds were used for relative calculation of ESBO. Furthermore, the confirmation patterns of monomeric plasticizers in standard mixture Set I was presented in transesterification chromatogram (Figure 4.4B) as follows: DMP_{IS} changed to diethyl pimelate (DEP_{IS}). DEHP reforms to 2-ethyl hexanol (Ehol), diethyl phthalate (DEP) and a part of incomplete ethylhexyl ethyl phthalate (EHEP). DIDP converted to isodecyl ethylhexyl phthalate (IDEP) and liberated isodecanol (10-ol) and contribution of DEP. For 2-EHA as an acid compound that did not react in transesterification and then was absent. For EN, OA and EA were resisted the transformation showing the retention time to be the same with direct analysis.

Figure 4.5A, the chromatogram of standard mixture Set II on the direct analysis shows DBS, ATBC, DEHA and DINCH, respectively. The chromatogram of transesterification (Figure 4.5B) shows the confirmation pattern of monomeric plasticizers as follows: DBS converted to diethyl sebacate (DES), DEHA changed to diethyl adipate (DEA) and also 2-ethyl hexanol (Ehol). Transesterification of DINCH is extremely slow, only a small amount converted to isononyl ethyl cyclohexane-1,2-dicarboxylate (INEHC) and diethyl cyclohexane-1,2-dicarboxylate (DEHC) and liberated isononanol (9-ol). The two peaks of stereoisomer of DINHC were distinguishable from DINP (as described below). ATBC and TAC were absent in the transesterification chromatogram.

Figure 4.6A, the chromatogram of standard mixture Set III on the direct analysis reveals acPG and DINP. The PAs present in the transesterification chromatogram (Figure 4.6B) were converted to diethyl adipate (DEA) the same as in transesterification form of DHEA because PAs was mixture compound of adipic acid and propanediol, terminated with octanol and decanol. In addition, transesterification able confirmation of acPG become lauric (C12:0) and DINP converted to DEP and isononanol (9-ol) the same as DINCH.

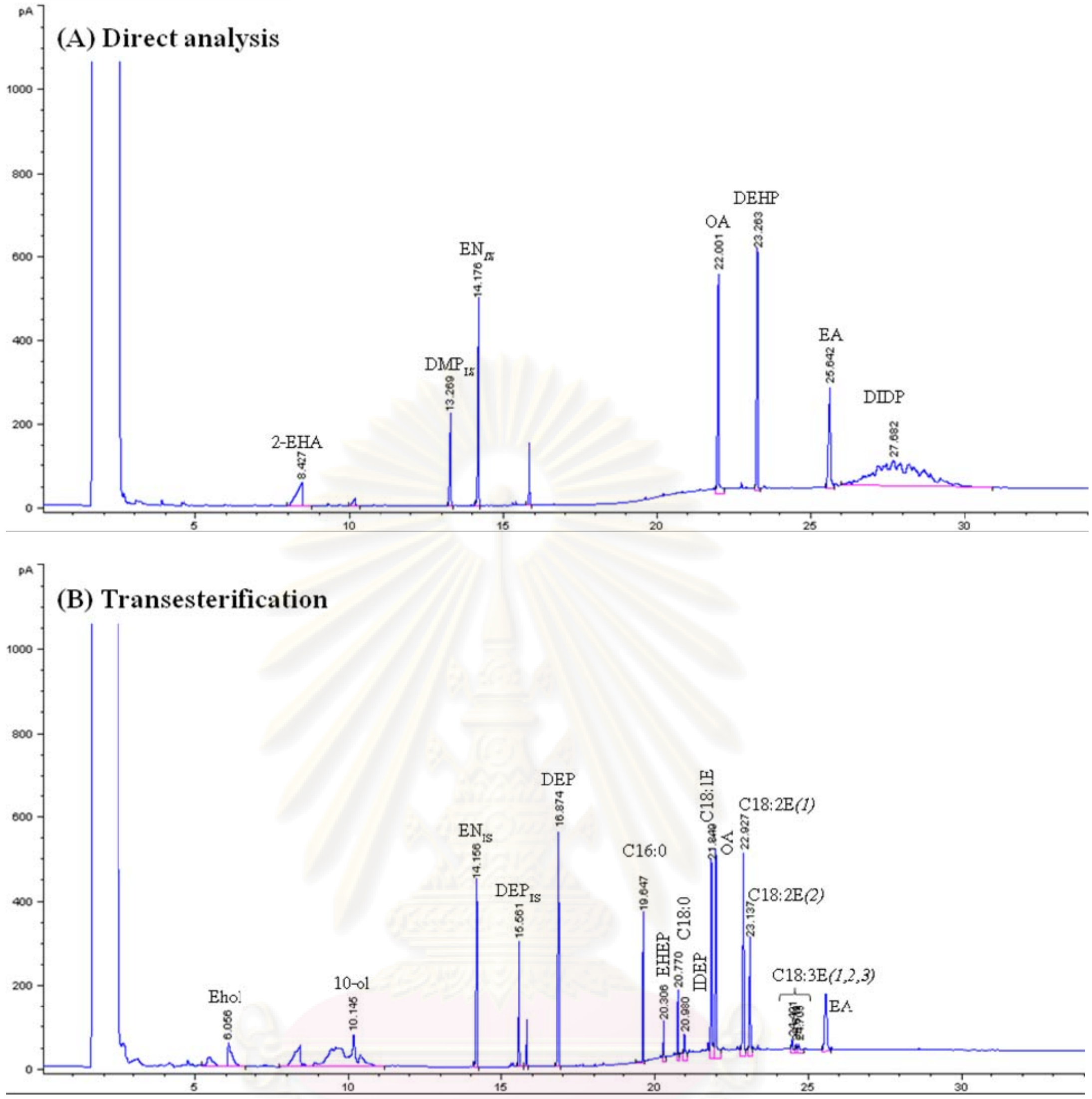


Figure 4.4 Chromatogram of the standard mixture Set I by the GC conditions listed in Table 4.3: (A) direct analysis, (B) transesterification.

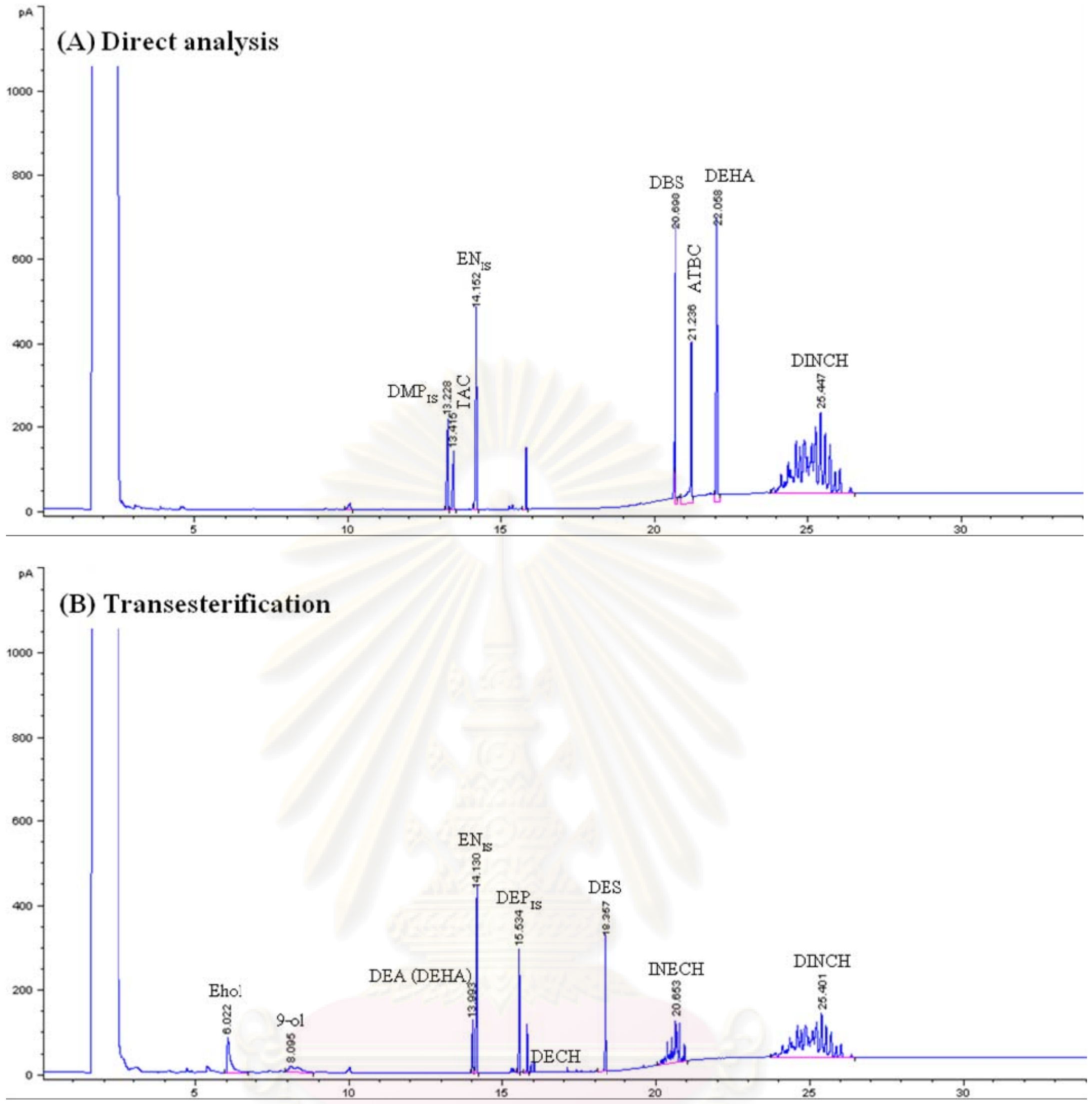


Figure 4.5 Chromatogram of the standard mixture Set II by the GC conditions listed in Table 4.3: (A) direct analysis, (B) transesterification.

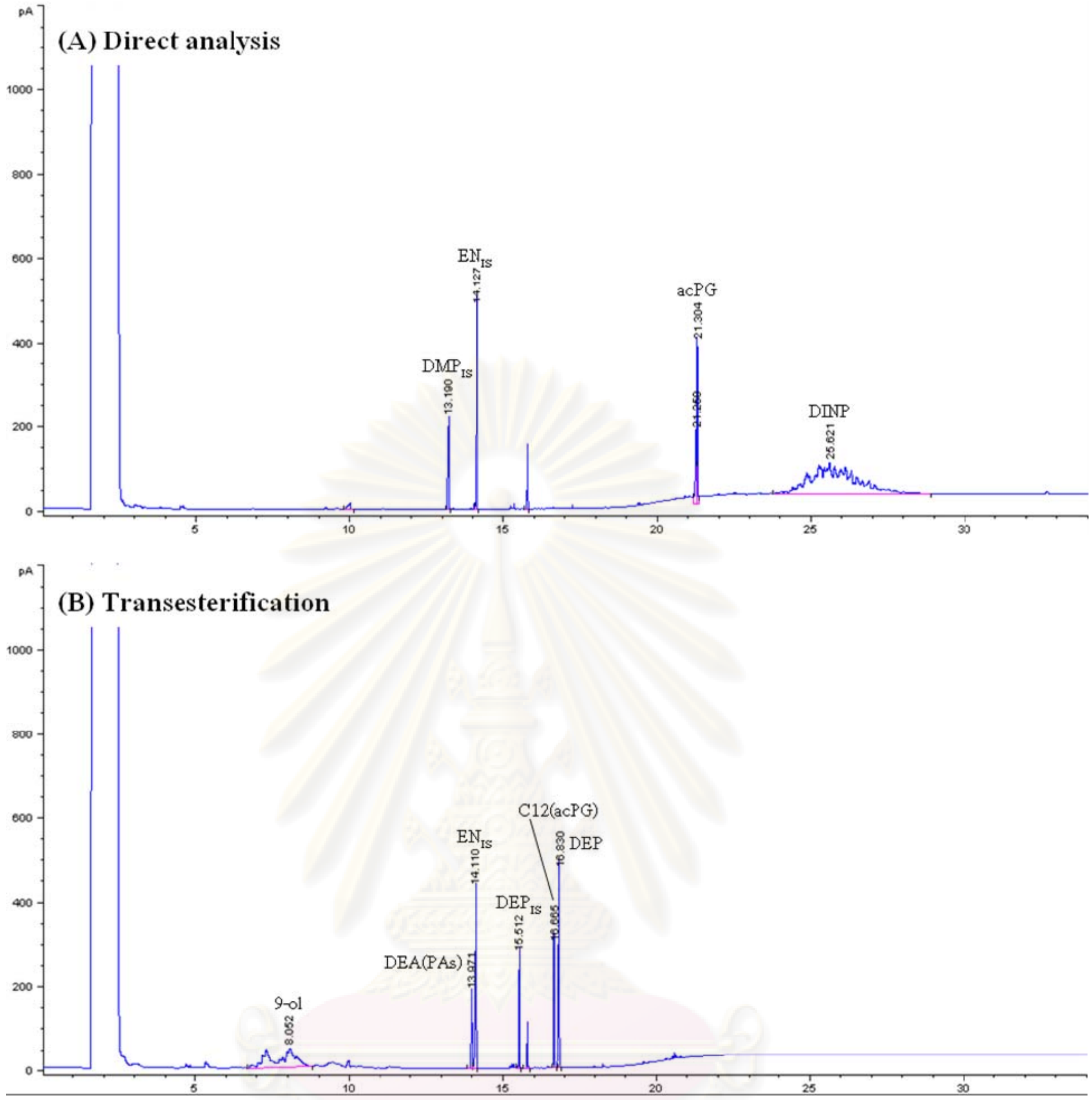


Figure 4.6 Chromatogram of the standard mixture Set III by the GC conditions listed in Table 4.3: (A) direct analysis, (B) transesterification.

4.1.2.2 Response Factor of Plasticizer

Response Factor (RF) relating to the response of each analyte under given GC condition in Table 4.3, the peak area of substance divided by the weight of substance injected as described in Equation (10). For monomeric plasticizers, DMP_{IS} used as internal standard for quantitative calculation in direct analysis. As well as DEP_{IS} was used for quantitative of ESBO and PAs in transesterification. Table 4.4 showed the response factor value of standard plasticizers.

$$\text{Response Factor (RF)} = \frac{(pA_{IS} \times m_{plasticizers})}{(m_{IS} \times pA_{plasticizers})} \qquad \ldots\ldots. (10)$$

where; pA_{IS} is peak area of internal standard, m_{IS} is mass of internal standard (mg), $pA_{plasticizer}$ is peak area of standard plasticizer, $m_{plasticizer}$ is mass of standard plasticizer (mg).

Table 4.4 The response factor of plasticizers referring to DMP_{IS} and DEP_{IS} as internal standard.

Plasticizer	Response Factor	
	DMP_{IS}	DEP_{IS}
acetyl partial (acPG)	0.92	
acetyl-*tri*-butyl citrate (ATBC)	1.36	
dibutyl sebacate (DBS)	0.73	
di-(2-ethylhexyl) adipate (DEHA)	0.66	
di-(2-ethylhexyl) phthalate (DEHP)	0.57	
diisodecyl phthalate (DIDP)	0.63	
diisononyl-1,2-cyclohexane dicarboxylate (DINCH)	0.66	
diisononyl phthalate (DINP)	0.63	
epoxidized soybean oil (ESBO)		1.09
erucamide (EA)	0.80	
oleamide (OA)	0.58	
polyadipates (PAs)		1.36
triacetin (TAC)	1.41	

4.1.2.3 Composition of Gasket

The quantitative analysis of plasticizers in the gasket were performed by response factor as followed by Equation 11 and 12 and Table 4.5 showed the components of plasticizers in lug cap 48 and 63 mm, respectively.

$$\text{Concentration of plasticizer (\%)} = \left(\frac{m_{IS} \times pA_{plasticizer} \times RF}{m_{sample} \times pA_{IS}}\right) \times 100 \qquad \ldots\ldots (11)$$

$$\text{Original amount of plasticizers (mg)} = \frac{\text{\% of plasticizers} \times \text{weight of gasket (g)}}{100} \times 1000$$

$$\ldots\ldots (12)$$

where; m_{IS} is mass of internal standard (mg), $pA_{plasticizer}$ is peak area of plasticizers, RF is the response factor of plasticizers, m_{sample} is weight of gasket sample (mg), pA_{IS} is peak area of internal standard.

Table 4.5 The quantitative composition of plasticizers in gasket in term of g/100g of lug cap 48 and 63 mm.

Gasket	Original amount of plasticizer (mg)				
	TAC	DBS	DINCH	ESBO	Total
lug cap 48 mm No.1	110.9	44.6	132.7	250.5	538.6
lug cap 48 mm No.2	110.9	44.6	133.7	242.6	531.6
lug cap 48 mm No.3	110.9	44.6	136.6	227.7	519.8
lug cap 48 mm No.4	132.7	44.6	132.7	249.5	559.4
lug cap 48 mm No.5	132.7	44.6	133.7	246.5	551.4
lug cap 48 mm No.6	132.7	44.6	133.8	219.8	540.5
average	121.8 ± 11.9	143.7 ± 0.0	133.8 ± 1.5	239.4 ± 12.7	540.2 ± 14.1
lug cap 63 mm No.1	261.3	133.9	163.8	269.4	855.4
lug cap 63 mm No.2	278.2	133.9	182.0	301.6	897.0
lug cap 63 mm No.3	258.7	130.0	163.8	289.9	845.0
lug cap 63 mm No.4	364.0	178.1	250.9	339.3	1132.3
lug cap 63 mm No.5	260.0	144.3	189.8	308.1	902.2
lug cap 63 mm No.6	257.4	141.7	187.2	328.9	915.2
average	279.9 ± 41.9	143.7 ± 17.7	189.6 ± 32.1	310.7 ± 19.4	924.5 ± 105.5

Figure 4.7 – Figure 4.8 showed the chromatograms of gasket sample from lug cap 48 mm and lug cap 63 mm, respectively. The result showed both samples were composed of the same type of plasticizers, ESBO, TAC, DBS and DINCH. The quantify of each plasticizers in lug cap 48 mm and lug cap 63 mm are slightly different as showed in Table 4.5.

The ESBO was a principle of plasticizers in both gaskets. ESBO is used as plasticizers as well as stabilizer for PVC, due to its epoxy content minimizes the autocatalytic deterioration by the scavenger hydrogen chloride which is released when PVC receives heat treatment [9]. Other plasticizers added for improved oil resistance and provided low temperature performance and low volatility properties of gasket.

The quantitative information of plasticizers in gasket by using ATR FT-IR microspectroscopy and GC-FID indicated the same results. It composed of ESBO, TAC, DBS and DINCH. However, ATR FT-IR microspectroscopy was a qualitative method which is suitable for the screening of plasticizers in the gasket while GC-FID was a qualitative and quantitative method for identification gasket composition. Therefore, GC-FID was suitable for study of gasket composition that made us known types and original amount of plasticizers in the gasket potentially migrate into fatty food simulant that important information prior to study the migration of plasticizers by simulation in the next step.

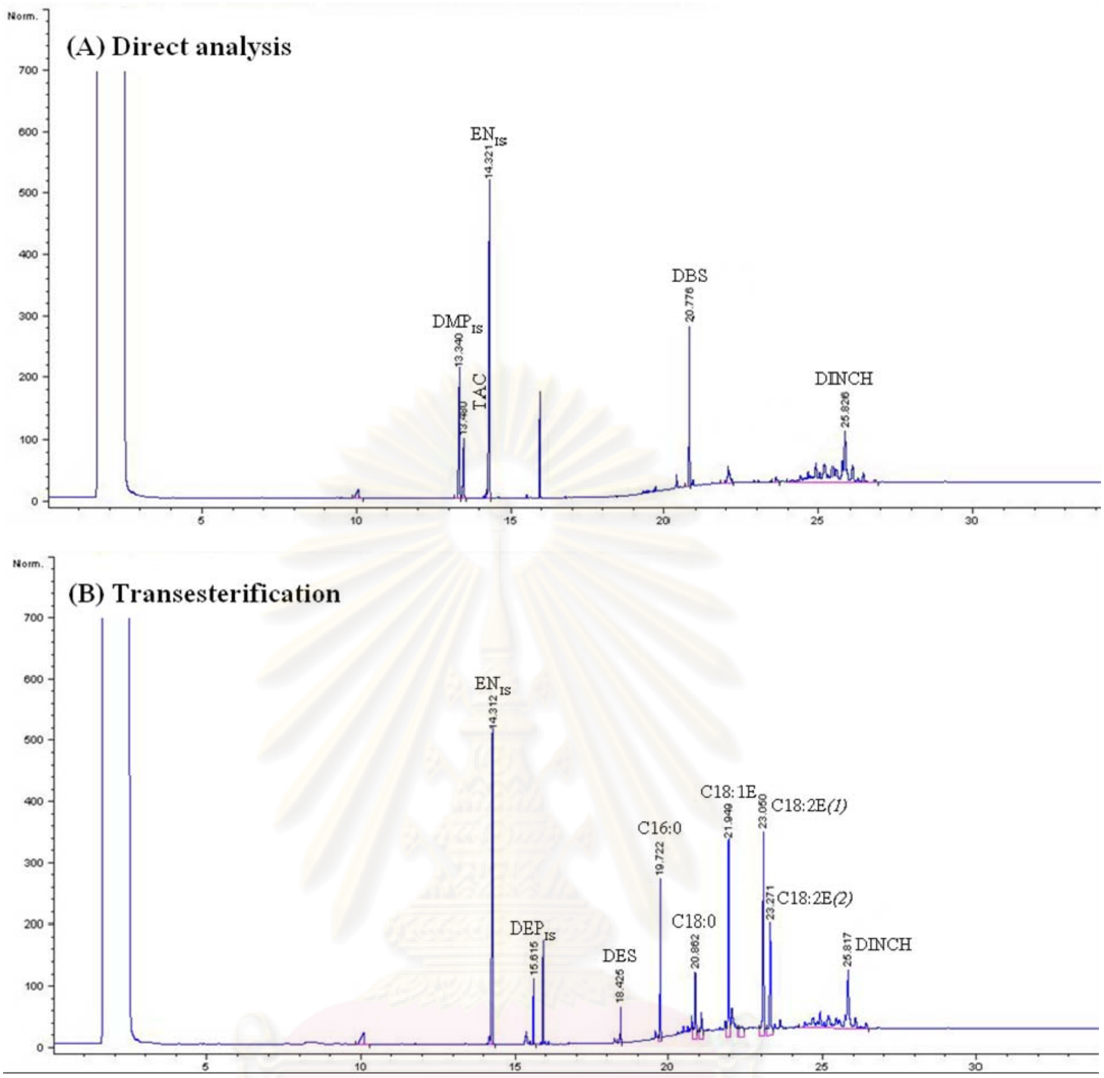


Figure 4.7 Chromatogram of gasket composition from lug cap 48 mm by the GC conditions listed in Table 4.3: (A) direct analysis, (B) transesterification.

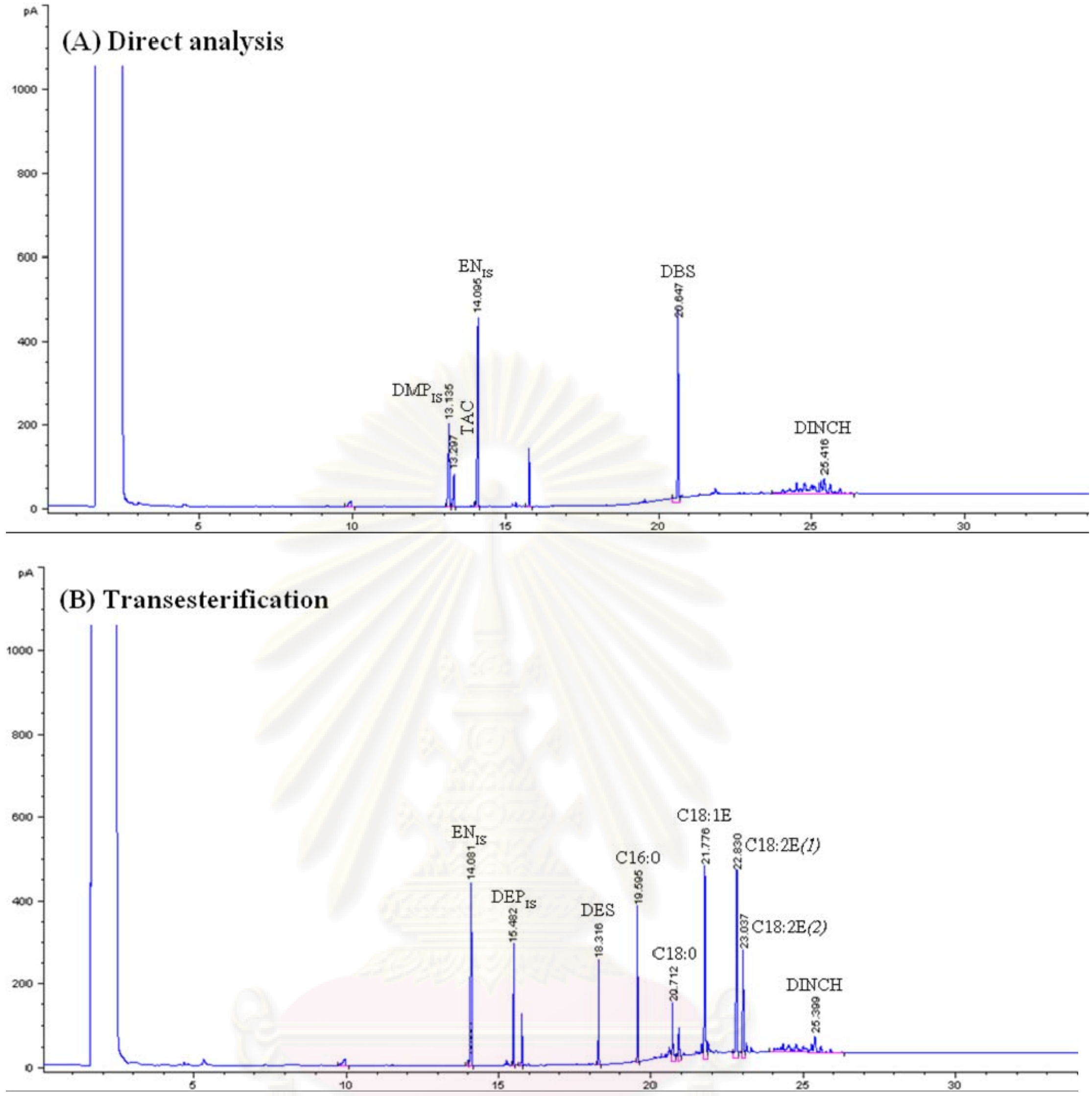


Figure 4.8 Chromatogram of gasket composition from lug cap 63mm. by the GC conditions listed in Table 4.3: (A) direct analysis, (B) transesterification.

4.2 The Result of Plasticizers Migration into Fatty Food Simulant

4.2.2 ATR FT-IR microspectroscopy

In this study, the migration testing was performed using simulation procedure in olive oil (fatty food simulant). The gasket sample after simulation was studied by ATR FT-IR microspectroscopy in the same way as the previous studied in 4.1.1. Figure 4.9 showed ATR spectra of gasket after simulation carried out by normal mode, contact-and-collect, and absorbance enhancement.

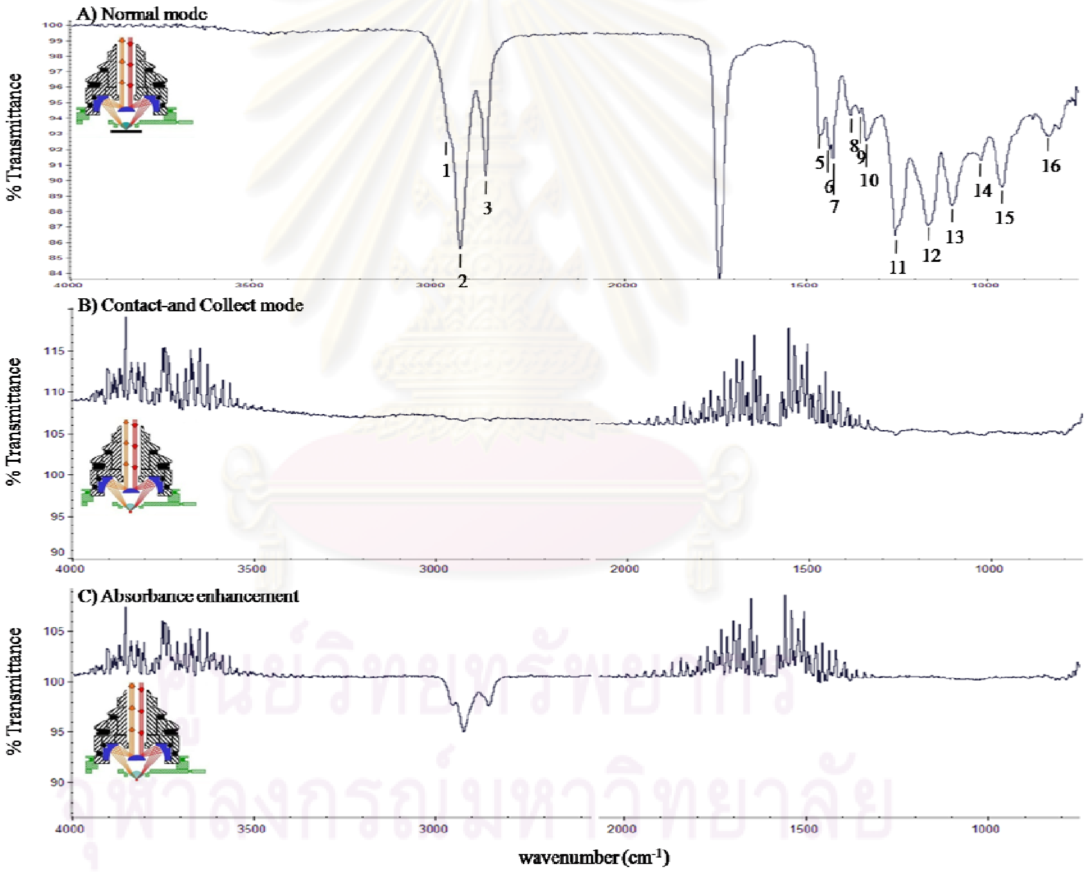


Figure 4.9 ATR spectra of gasket after simulation acquired by three modes of sample collection; (A) Normal mode, (B) Contact-and-Collection mode and (C) Absorbance Enhancement.

The observed ATR spectrum of normal operation that showed the identical spectra with previous work of a new gasket sample (Figure 4.1). An ATR spectrum was identified of plasticizers in the gasket after simulation such as ESBO, TAC, DBS and DINCH. On the other hand, ATR spectra of contact-and-collect and absorbance enhancement were absent. This is because the contact-and-collect operation was a sampling technique for identification of trace elements at the surface of the sample, the absent spectra showed the plasticizers which were at the surface of old gasket sample had been transferred into the fatty food simulant already. However, the plasticizers in the next layer remained in the gasket as showed in ATR spectra of normal operation.

4.2.3 ESBO Analysis in Fatty Food Simulant

Injection of 1 µL was used for all samples. The optimized GC condition is reported in Table 4.6 and Figure 4.10 showing the chromatogram of diastereoisomers of methyl diepoxy linoleate as quantification analyte of ESBO and methyl diepoxy eicosanoate as internal standard.

Table 4.6 Gas chromatography-Mass spectrometry conditions for ESBO analysis in fatty food simulant

GC-MS Parameters	GC-MS Conditions
Analytical column	Rtx-2330, 15 m × 0.25 mm i.d. × 0.20 µm
Oven temperature	Initial 110 °C for 1 min, 20 °C/min to 200 °C, 8 °C/min to 260 °C hold 2 min.
Injection mode	splitless
Injection temperature	300 °C
Carrier gas	Helium, 3mL/min
Detector	Mass Spectrometer, SIM mode
SIM fragment	*m/z* 155 for methyl diepoxy linoleate (referred ESBO) *m/z* 183 for methyl diepoxy eicosanoate (referred internal standard)
Auxiliary temperature	300 °C

The analysis of ESBO into fatty food simulant carried out by transesterification direct into olive oil. For transesterification, the triglycerides of olive oil were possibility convert to methyl ester as same as ESBO. Therefore, the methyl diepoxy linoleate was a quantify analyte of ESBO due to it was high amount in the ESBO (as a small amount in olive oil). Furthermore, two peaks of methyl diepoxy linoleate and internal standard were separated from other fatty acid methyl esters with no potential interference from other compounds. The peak ratio of C18:2E (*1*) and C18:2E (*2*) was 2:1 and these two peaks were the same as masses, and the same occurred for the internal standard.

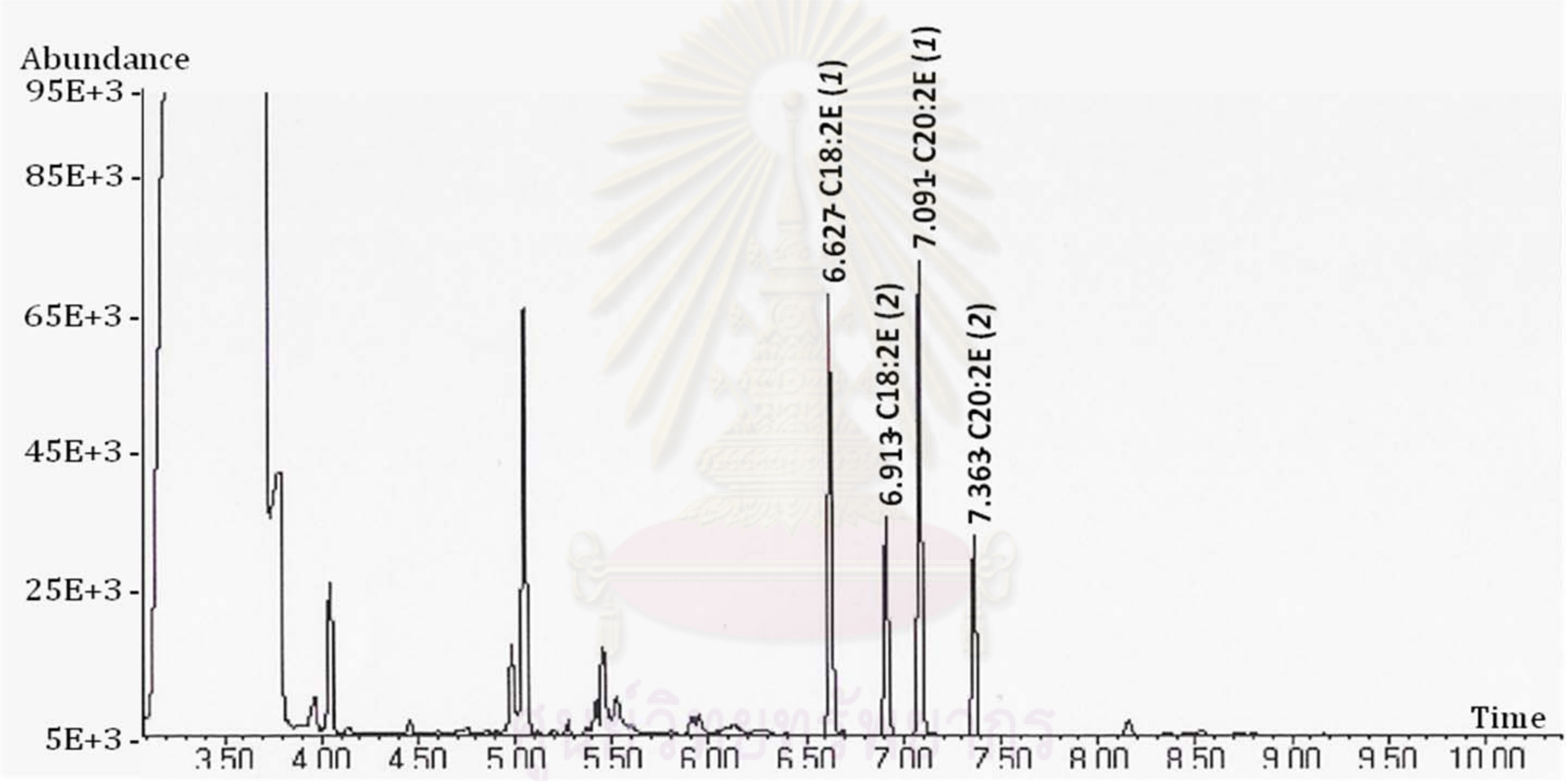


Figure 4.10 The total ion chromatogram of methyl diepoxy linoleate [C18:2E (*1*), C18:2E (*2*)] and methyl diepoxy eicosanoate [C20:2E (*1*), C20:2E (*2*)], from standard mixture by SIM mode of the condition listed in Table 4.6.

4.2.3.2 Response Factor of ESBO

The response factor of ESBO was calculated according to by Equation 13. For the calculation of response factor was acquired two values of the first analogue of (C18:2E (*1*)/C20:2E (*1*)) and the second analogue of (C18:2E (*2*)/C20:2E (*2*)) referred to RF1 and RF2, respectively were acquired.

$$\text{Response Factor (RF)} = \frac{pA_{\text{std.C18:2E}}}{pA_{\text{std.C20:2E}}} \qquad \text{……. (13)}$$

Where; $pA_{\text{std.C18:2E}}$ is peak area of standard of methyl diepoxy linoleate, $pA_{\text{std.C20:2E}}$ is peak area of standard of methyl diepoxy eicosadienoate.

The results of response factor were 1.05 and 0.90 of the RF1 and RF2, respectively.

4.2.1.2 The Quantitative of ESBO in Fatty Food Simulant

The concentration of ESBO in the food simulant was calculated according to Equation 14 and 15. The average of the first of analogue of methyl diepoxy linoleate and the second of analogue of methyl diepoxy linoleate referred to ESBO in fatty food simulant.

$$\text{Concentration of ESBO (mg/kg)} = \left(\frac{m_{IS} \times pA_{ESBO} \times RF}{m_{sample} \times pA_{IS}}\right) \times 10^6 \qquad \text{…… (14)}$$

$$\text{amount of ESBO (mg)} = \frac{\text{concentration of ESBO (mgkg}^{-1}\text{)} \times \text{weight of food simulant (g)}}{1000} \qquad \text{…… (15)}$$

Where; m_{IS} is mass of internal standard (mg), pA_{ESBO} is peak area of methyl diepoxy linoleate, RF is response factor of ESBO, m_{sample} is weight of fatty food simulant (mg), pA_{IS} is peak area of methyl diepoxy eicosadienoate.

4.2.4 Monomeric Plasticizer Analysis in Fatty Food Simulation

The injection of 2 μL was used for all samples. The optimized GC condition was reported in Table 4.7 and Table 4.8 showing mass-to-charge ratio (*m/z*) of monomeric plasticizers and internal standard. The chromatogram of standard mixture of monomeric plasticizers was shown in Figure 4.12.

Table 4.7 Gas chromatography-Mass spectrometry condition for monomeric plasticizer analysis in fatty food simulant

GC-MS Parameters	GC-MS Conditions
Pre-column	DB-1, 0.5 m × 0.25 mm i.d. × 0.1 μm
T-piece connector	Fused silica tubing, 13 cm × 0.25 mm i.d. × 0.20 μm
Analytical column	Rtx-225, 15 m × 0.25 mm i.d. × 0.20 μm
Carrier gas	Helium, 5 mL/min
Injection temperature	60 °C
Injection mode	PTV, splitless
Liner	2 mm i.d., glass liner with plug of glass wool at 6 cm
PTV injector	Initial 60 °C for 0.2 min, 14.5 °C/sec to 250 °C, 14.5 °C/sec to 350 °C for 5 min.
Oven temperature	Initial 90 °C for 4 min, 25 °C/min to 190 °C, 15 °C/min to 250 °C hold 10 min.
Detector	Mass Spectrometer, SIM mode (as showed in Table 4.9)
Auxiliary temperature	300 °C

Table 4.8 The mass-to-charge ratio (*m/z*) used for SIM mode

Compound	Type of compound	SIM fragment
Triacetin (TAC)	plasticizers	145
Diheptyl phthalate (DHP)	internal standard	149
Diisononyl cyclohexane-1,2-dicarboxylate (DINCH)	plasticizers	155
Dipropyl sebacate (DPS)	internal standard	185
Dibutyl sebacate (DBS)	plasticizers	185

4.2.4.2 Programmed Temperature Vaporizing

Programmed Temperature Vaporizing (PTV) is an injection technique for separation monomeric plasticizers from fatty food matrix during injection. PTV is a variable injector temperature consisting of three steps (as shown in Figure 4.11) which are deposition of sample liquid, desorption of solutes and heating out of bulk of oil.

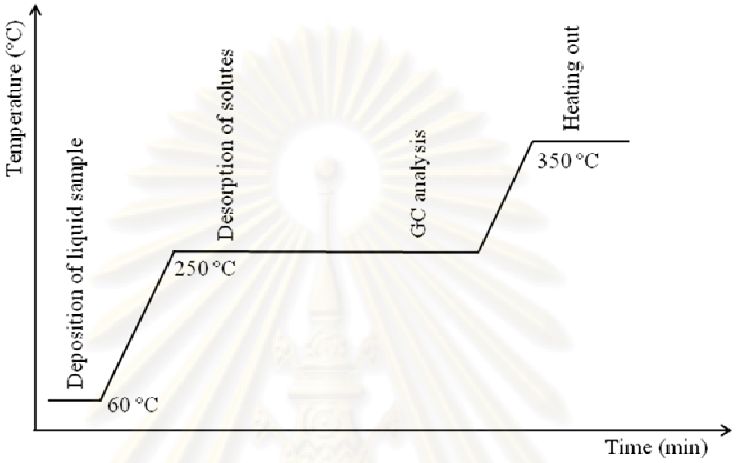


Figure 4.11 Schematic presentation of temperature program of the PTV injector

Step 1: Low initial injector temperatures facilitated the liquid sample deposited onto a plug of glass wool and prevented rapid evaporation of solvent and sample. Furthermore, the tip of syringe needle can be properly place the liquid sample on the plug of glass wool to make evaporation of solutes more efficient.

Step 2: For desorption step, the temperature should be kept as low as possible in order to avoid evaporation of the bulk of oil components. Desorption temperature at 250 °C was high enough for plasticizers to evaporate and can be completely transferred to pre-column and analytical column.

Step 3: When the solutes completely transferred to the analytical column, the switching valve redirected the carrier gas pass through the T-piece connector as well as the flow backflushing the pre-column into the injector at the same time with the injector chamber was heating out in order to prevent bulk of oil transferring to the column. The vaporizing chamber of the injector was heated up to 350 °C for 5 minutes during the heating up step, the carrier and vapor of oil were venting out to split outlet.

Figure 4.12 showed the chromatogram of five peaks of standard monomeric plasticizers and internal standard. The DHP as internal standard was used for calculation quantity of TAC and DINCH as well as DPS as internal standard used calculation quantity of DBS.

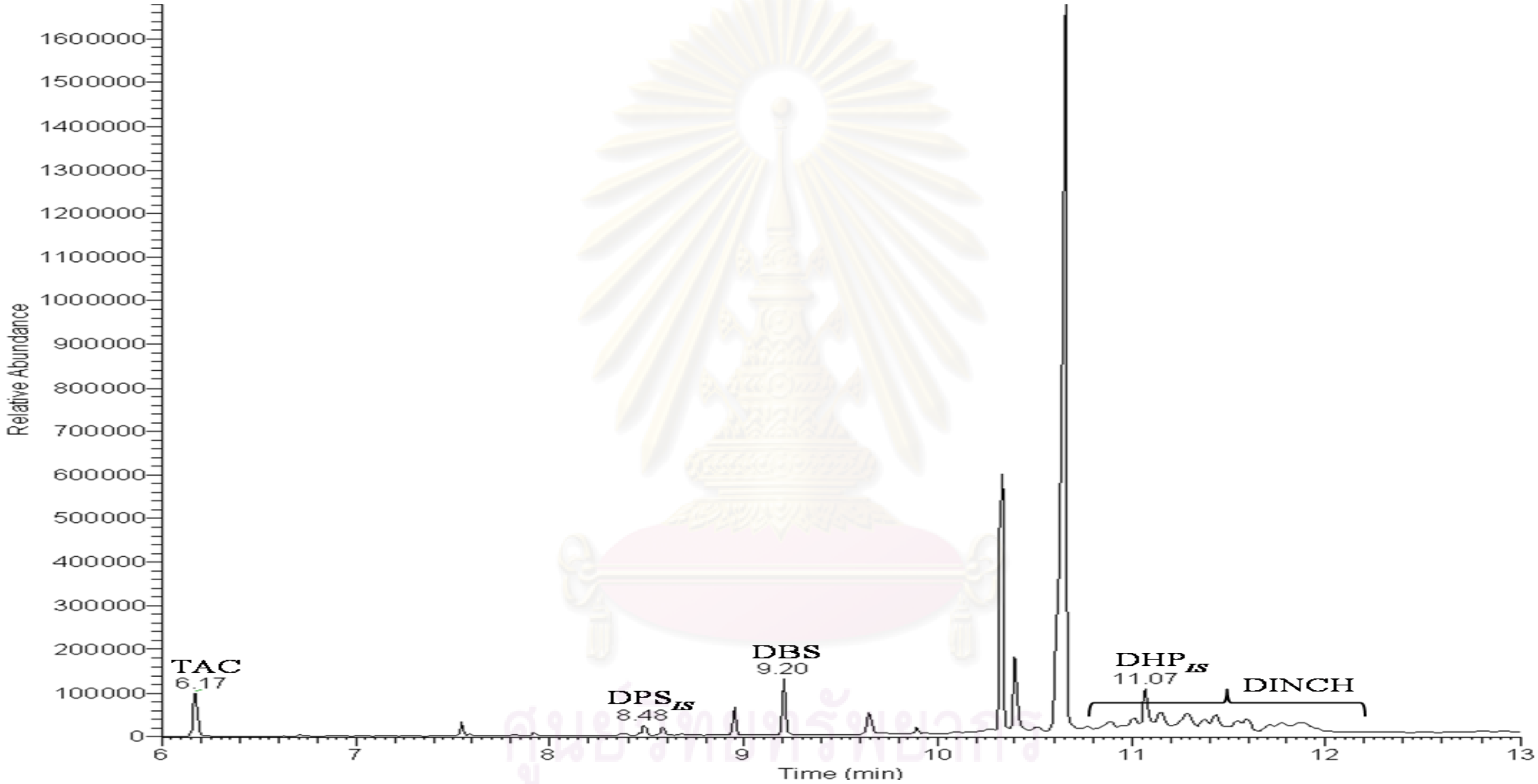


Figure 4.12 The total ion chromatogram of TAC, DBS, DINCH, DHP and DPS from standard mixture by using SIM mode of the condition listed in Table 4.7.

4.2.4.3 The Standard Calibration Curve

The determination of monomeric plasticizers was carried out by a standard calibration curve of three analytes (i.e. TAC, DBS and DINCH) from 1.5 – 400 mg/kg using GC condition followed in Table 4.7.

From plots in APPENDIX D, the linear relations were obtained. The coefficients of determination (R^2) of all studied substances are greater than 0.9980 for all standards, confirming strong linear relations. The calibration curve of each analytes were prepared from 9 concentration levels (triplicate analyses), Table 4.9 showed a good linear model. The slopes of analytes were shown that the sensitivity of DBS is higher than DINCH and TAC, respectively.

Table 4.9 The coefficient of determination (R^2) of TAC, DBS and DINCH in ranges of 1.5 – 400 mg/kg of 9 points and triplicate analyses.

Compound	Replication	t_R (min)	Slope	y-Intercept	R^2
TAC	1	6.24	0.0063	0.0130	0.9986
	2	6.39	0.0715	0.4430	0.9983
	3	6.32	0.0099	0.0425	0.9999
DBS	1	9.26	0.3071	-2.3371	0.9977
	2	9.38	0.1310	-0.3120	0.9997
	3	9.32	0.1642	0.1769	0.9998
DINCH	1	11.23	0.1941	-0.0200	0.9996
	2	11.41	0.0681	-0.0961	0.9997
	3	11.32	0.1634	0.1643	0.9998

4.2.5 Parameters Affecting The Migration of Plasticizers into Fatty Food Simulant

Migration testing is usually carried out on the food simulant than food themselves. Foods are complex mixtures of variable compositions and their analyses can have many complexities in both analysis and practical aspects. Therefore, food simulant have been used for a simplified migration study and also studied by simulation under desired conditions.

The olive oil was commonly used as the fatty food simulant as also in this study. It was purchased from a supermarket in Bangkok. Refined olive oil is a food grade olive oil, obtained by refined virgin olive oil whereby no solvents have been used in the extraction process, but rather has been refined with the use of charcoal and other chemicals and physical filters. It can also be equivalent to "pure olive oil"

The ATR FT-IR microspectroscopy and GC-FID were used to identify qualitative analysis of plasticizers in the new gasket. The results of two methods were identical, they consist of ESBO, TAC, DBS and DINCH. GC-FID was able to quantify the total amount of plasticizers, in lug cap 48 mm and lug cap 63 mm as 540.2 ± 14.1 and 924.5 ± 105.5 mg, respectively. Furthermore, ESBO was main plasticizers in gasket samples of both types.

Both lug caps were used for studying of migration for each plasticizer into fatty food simulant (olive oil). The migration of each plasticizers in the gasket (such as TAC, DBS, DINCH and ESBO) were performed in triplicates for both lug caps according to the parameters of contact time, contact temperature, the initial heating of pasteurization and contact surface area which described in Experiment Section 3.5.1.

The results of ATR FT-IR microspectroscopy of gasket after simulation showed that no any spectra of plasticizers at the surface of the gasket which acquired from contact-and-collect and absorbance enhancement operations as described in 4.2.1. These results were indicated the plasticizers were transferred from the gasket into fatty food simulant. The analysis of ESBO in the fatty food were performed in Experiment Section 3.5.2 followed by GC-MS as in Table 4.6 as well as the analysis of monomeric plasticizers (such as TAC, DBS and DINCH) in the fatty food were performed in Experiment Section 3.5.3 followed by GC-MS as in Table 4.7 by PTV-GC-MS.

The amount of each plasticizer that migrated into fatty food simulant according to the studied parameters are identified by GC as followed.

4.2.5.2 The Effect of Contact Time

The influence of the duration time on the migration of plasticizers into the fatty food simulant was investigated by inverting the jar which contained the fatty food simulant at 30 °C for 30 days. The contact time was studied during 6 hours, 5 days, 10 days, 15 days and 30 days, respectively. The jars were shaken prior to analysis in order to let the oil adhering to the gasket run down into the whole content of food and for homogenizing food simulant.

The results of each plasticizer migrated into fatty food simulant were shown in Table 4.10, both lug caps showed the plasticizers continuously migrated into fatty food simulant corresponding to the contact times. ESBO showed the highest migration and the monomeric plasticizers showed less migration of TAC, DBS, DINCH, respectively. The increasing migration can be explained because of the interface of gasket/fatty food simulant, the plasticizers were transferred from the gasket by the diffusion process. Also the plasticizers were extracted and dissolved by the fatty food simulant as well as remaining adhered to the gasket until the exchange of fatty food simulant at the interface, the plasticizers primarily transferred stepwise from the surface of the gasket into the fatty food simulant.

Figure 4.13 – Figure 4.14 showed the potential of each plasticizer which migrated into the fatty food simulant for both lug caps. At 30 days, ESBO migrated up to 31 times for lug cap 48 mm and 40 times for lug cap 68 mm. The migration of ESBO can be understood because ESBO is a triglyceride of fatty acids and the properties resemble the fatty acids in the olive oil so ESBO easily dissolve in a fatty food simulant (olive oil) which follows the rule of “like dissolves like”. Also because ESBO has the

highest original amount in the gasket, the increasing of extracted ESBO in the fatty food simulant appeared. The migration of monomeric plasticizers showed the relation of the size of plasticizers molecule on the migration into fatty food simulant. The small molecule of TAC had a high migration compared with the large molecule of DBS and DINCH, respectively. Because the small molecule moved easily and transferred to the interface of gasket/fatty food simulant, the extracted monomeric plasticizers increased migration into the fatty food simulant.

Furthermore, the structure of plasticizer molecule related to the migration of plasticizers. DBS as the linear molecule (Figure 4.2D) had a higher migration than DINCH as branched molecule (Figure 4.2C), because the branched molecule hindered movement of DINCH to the interface of gasket/fatty food simulant making its migration slow.

Therefore, the contact time of the gasket and fatty food simulant, the original amount of plasticizers, size and the structure of plasticizers were affected on the migration of plasticizers into fatty food.

Table 4.10 The effect of contact time has influence on the migration of plasticizers from lug cap 48 and 63 mm

Metal lid sample	Contact time (days)	Amount of plasticizer migrating into the fatty food simulant (mg)			
		TAC	DBS	DINCH	ESBO
Lug cap 48 mm	6 hrs.	0.88 ± 0.36	0.17 ± 0.05	0.08 ± 0.01	0.87 ± 0.01
	5	0.97 ± 0.29	0.32 ± 0.15	0.13 ± 0.00	11.24 ± 2.88
	10	1.06 ± 0.27	0.49 ± 0.11	0.19 ± 0.12	15.69 ± 0.15
	15	1.24 ± 0.18	0.63 ± 0.11	0.26 ± 0.06	18.92 ± 6.34
	30	1.55 ± 0.52	0.74 ± 0.10	0.33 ± 0.05	26.84 ± 0.78
Lug cap 63 mm	6 hrs.	1.11 ± 0.14	0.93 ± 0.05	0.14 ± 0.03	1.37 ± 0.24
	5	1.28 ± 0.13	1.40 ± 0.12	0.21 ± 0.05	15.98 ± 1.06
	10	1.37 ± 0.14	1.84 ± 0.08	0.26 ± 0.05	24.60 ± 1.73
	15	1.53 ± 0.23	2.73 ± 0.05	0.33 ± 0.03	41.48 ± 4.37
	30	1.86 ± 0.09	3.09 ± 0.24	0.46 ± 0.03	55.03 ± 7.01

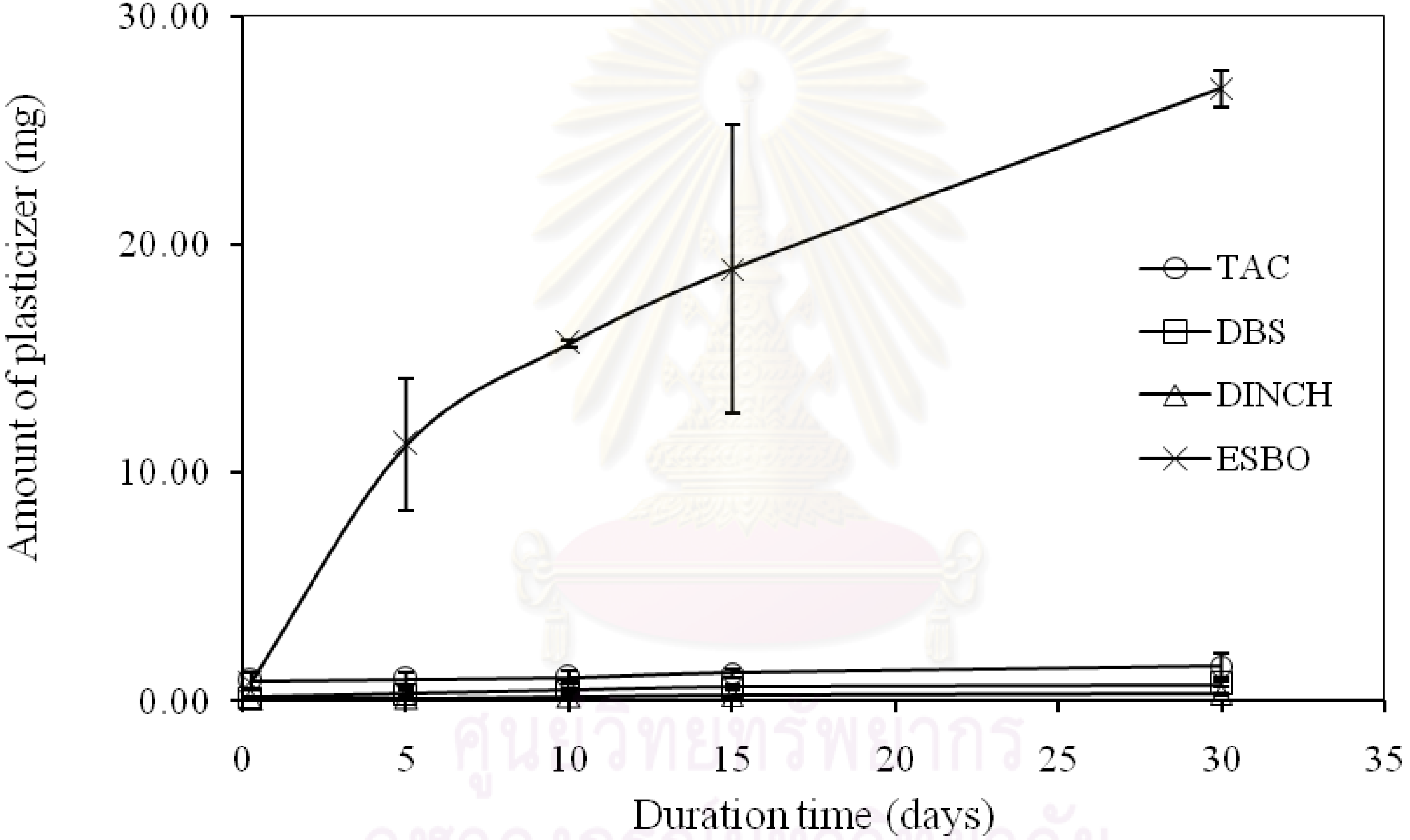


Figure 4.13 The potential migration of plasticizers conditioned of contact time for lug cap 48 mm

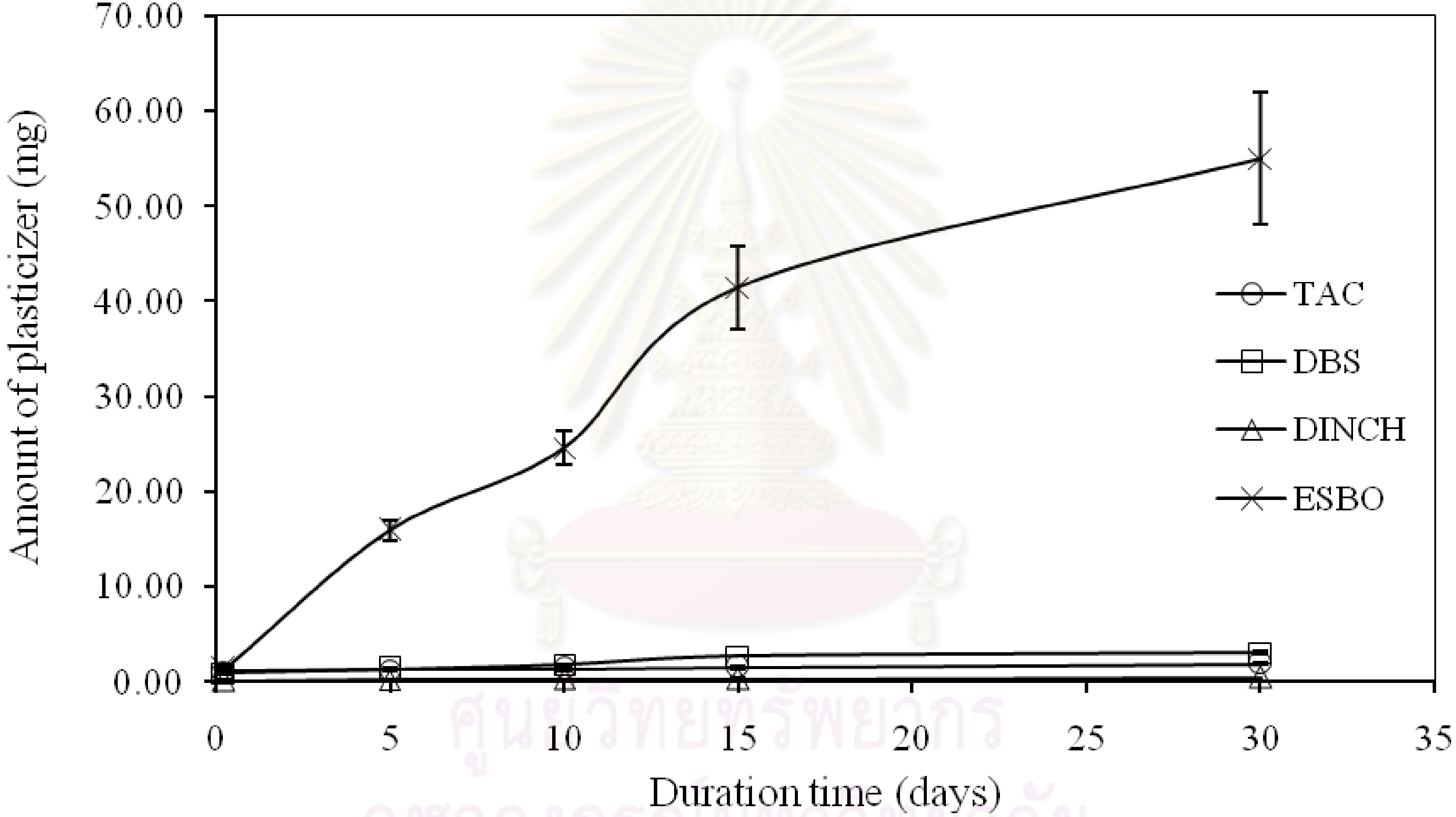


Figure 4.14 The potential migration of plasticizers conditioned of contact time for lug cap 63 mm

4.2.5.3 The Effect of Contact Temperature

The influence of the elevation contact temperature on the migration of plasticizers into the fatty food simulant was investigated from the jar which contained the fatty food simulant without initial heating which was upside down at 30 °C, 40 °C and 60 °C for 10 days, respectively.

The results of the migration of each plasticizer into fatty food simulant were shown in Table 4.11, both lug caps showed the relation of the higher migration on the elevation contact temperature. ESBO had the highest migration into fatty food simulant compared with monomeric plasticizers such as TAC, DBS, DINCH, respectively. The high temperature can accelerate the mobility of plasticize to move upward to the interface of gasket/fatty food simulant, the high amount of plasticizers at the surface were dissolved as well as extremely extracted by fatty food simulant, so the migration of plasticizers into fatty food simulant with higher amounts.

For the basic diffusion models, the migration should have increased by factors of approximately 10 each from 20 – 40 °C and from 40 – 60 °C [40]. However, Feigenbaum and co-workers explained the migrations of plasticizers from the gasket into food were far lower due to the more complex of migration mechanism and the various types of plasticizers in the gasket composition.

Figure 4.15 and Figure 4.16 showed the potential of plasticizers migrating into a fatty food simulant with the elevated contact temperature, for the migration of ESBO of lug cap 48 mm were increasing by factor of 1.9 from 30 – 40 °C and 1.7 from 40 – 60 °C. The migration of ESBO lug caps 63 mm were increasing by factors of 1.5 from 30 – 40 °C and 1.7 from 40 – 60 °C. The small of increasing factor as describes above. Furthermore, both graphs showed the high migration of ESBO because the high contact temperature increased the solubility of ESBO in the fatty food simulant and also can be described as in 4.2.4.1.

The migrations of TAC, DBS and DINCH depended on the size and the structure of the plasticizers. The migration of monomeric plasticizers can be described as follows in 4.2.4.1.

Table 4.11 The effect of contact temperature has influence on the migration of plasticizers from lug cap 48 and 63 mm

Metal lid sample	Contact temperature (°C)	Amount of migration plasticizer into fatty food simulant (mg)			
		TAC	DBS	DINCH	ESBO
Lug cap 48 mm	30	1.06 ± 0.27	0.49 ± 0.11	0.19 ± 0.12	15.69 ± 0.15
	40	1.19 ± 0.00	0.74 ± 0.11	0.24 ± 0.02	29.60 ± 8.57
	60	1.83 ± 0.53	1.03 ± 0.23	0.30 ± 0.02	49.22 ± 0.00
Lug cap 63 mm	30	1.37 ± 0.14	1.84 ± 0.08	0.26 ± 0.05	24.60 ± 1.73
	40	1.68 ± 0.12	2.36 ± 0.45	0.31 ± 0.01	36.28 ± 3.03
	60	2.15 ± 0.26	3.43 ± 0.22	0.43 ± 0.02	70.54 ± 0.95

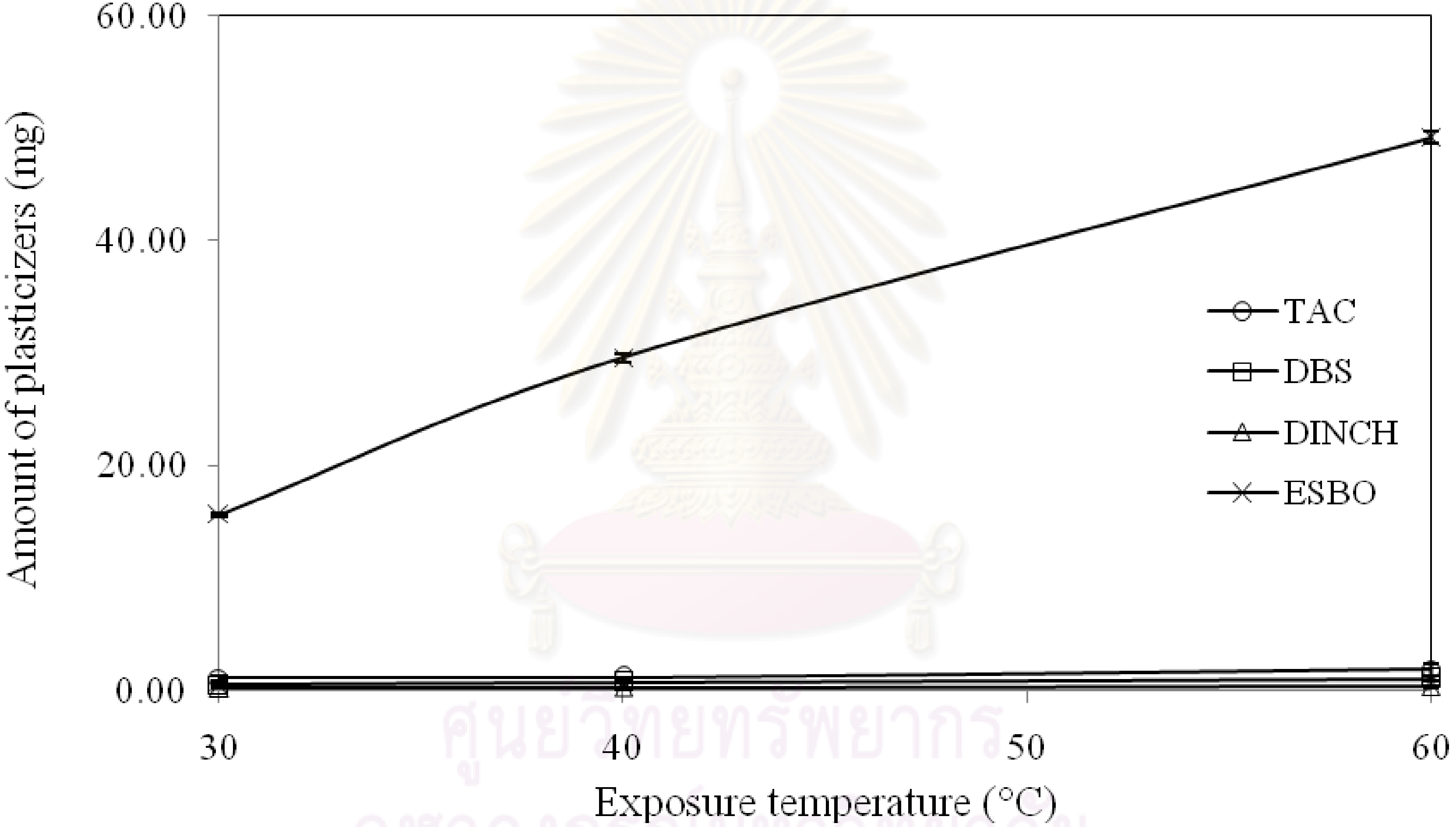


Figure 4.15 The potential migration of plasticizers conditioned of contact temperature for lug cap 48 mm

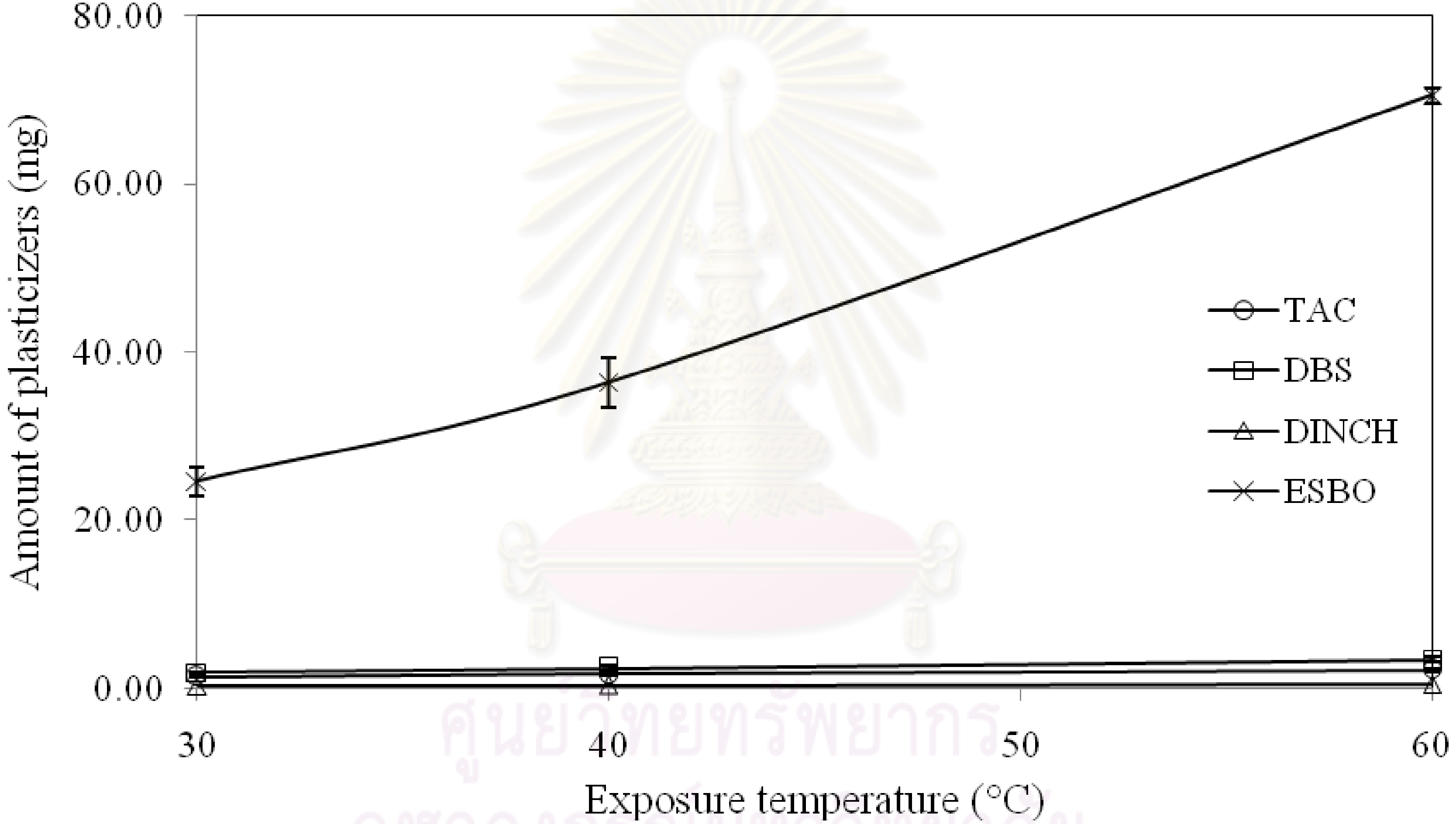


Figure 4.16 The potential migration of plasticizers conditioned of contact temperature for lug cap 63 mm

4.2.5.4 The Effect of Initial Heating

The initial heating used for the simulation of the pasteurization process which was heated to 100 °C for 1hour for inverted jar which contained the fatty food simulant. The influence of initial heating on the migration of plasticizers was performed by kept the inverted jar after initial heating process at 30 °C for 30 days. The migration of plasticizers was investigated during 6 hours, 5 days, 10 days, 15 days and 30 days, respectively for both lug caps.

The results of each of the plasticizers which migrated into the fatty food simulant of both lug caps were shown in Table 4.12 – Table 4.13, both data showing the migration of each plasticizer both with and without initial heating were compared. The migrations of each plasticizer with initial heating were lower than without initial heating. Also in the previous study, ESBO showed the highest migration into the fatty food simulant.

Figure 4.17 – figure 4.20 showed the potential of migration of ESBO, TAC, DBS and DINCH for lug cap 48 mm and Figure 4.21 – Figure 4.24 for lug caps 63 mm, respectively. The migrations of each plasticizer with initial heating were obviously distinguishable from the migration of plasticizers without initial heating. In a few days, the plasticizers showed a rapid migration into fatty food simulant by the influence of the high temperature from the initial heating process and the migrations were slow as well as no increasing migration appeared. Because the very high temperature of the pasteurization process (100 °C for 1hour) increasing the mobility of plasticizers upward to the interface of gasket/fatty food simulant. The high amount of plasticizers at the interface formed the de-plasticizers layer of PVC (100 – 200 μm) that blocked any further migration of plasticizers. The plasticizers from the next layer into the bulk of gasket were hindered which made the plasticizers slowly replenish to the interface of gasket/fatty food simulant, lead to minor migration of plasticizers.

For migration of ESBO at 30 days, the sample with initial heating of the lug cap 48 mm decreased 4.2 times and 3.7 times for lug caps 63 mm. For migration of TAC, DBS and DINCH very small amount of plasticizers appeared in the fatty food simulant. Furthermore, the migration of monomeric plasticizers depended on the size and the structure of plasticizers as described in 4.2.4.1.

Table 4.12 The effect of initial heating has influence on the migration of plasticizers from lug cap 48 mm

Metal lid sample	Duration (days)	Amount of migration plasticizer into fatty food simulant (mg)			
		TAC	DBS	DINCH	ESBO
Lug cap 48 mm (without initial heating)	6 hrs.	0.88 ± 0.36	0.17 ± 0.05	0.08 ± 0.01	0.87 ± 0.01
	5	0.97 ± 0.29	0.32 ± 0.15	0.13 ± 0.00	11.24 ± 2.88
	10	1.06 ± 0.27	0.49 ± 0.11	0.19 ± 0.12	15.69 ± 0.15
	15	1.24 ± 0.18	0.63 ± 0.11	0.26 ± 0.06	18.92 ± 6.34
	30	1.55 ± 0.52	0.74 ± 0.10	0.33 ± 0.05	26.84 ± 0.78
Lug cap 48 mm (with initial heating)	6 hrs.	1.09 ± 0.42	0.26 ± 0.03	0.09 ± 0.02	6.00 ± 1.30
	5	1.01 ± 0.39	0.21 ± 0.02	0.07 ± 0.03	6.20 ± 1.50
	10	0.92 ± 0.38	0.25 ± 0.03	0.07 ± 0.03	5.67 ± 0.15
	15	0.95 ± 0.30	0.28 ± 0.04	0.08 ± 0.03	5.87 ± 1.11
	30	0.89 ± 0.31	0.38 ± 0.09	0.06 ± 0.03	6.38 ± 0.33

Table 4.13 The effect of initial heating has influence on the migration of plasticizers from lug cap 63 mm

Metal lid sample	Duration (days)	Amount of migration plasticizer into fatty food simulant (mg)			
		TAC	DBS	DINCH	ESBO
Lug cap 63 mm (without initial heating)	6 hrs.	1.11 ± 0.14	0.93 ± 0.05	0.14 ± 0.03	1.37 ± 0.24
	5	1.28 ± 0.13	1.40 ± 0.12	0.21 ± 0.05	15.98 ± 1.06
	10	1.37 ± 0.14	1.84 ± 0.08	0.26 ± 0.05	24.60 ± 1.73
	15	1.53 ± 0.23	2.73 ± 0.05	0.33 ± 0.03	41.48 ± 4.37
	30	1.86 ± 0.09	3.09 ± 0.24	0.46 ± 0.03	55.03 ± 7.01
Lug cap 63 mm (with initial heating)	6 hrs.	0.98 ± 0.06	0.73 ± 0.19	0.10 ± 0.03	8.50 ± 0.90
	5	1.00 ± 0.09	0.80 ± 0.13	0.10 ± 0.02	9.10 ± 0.90
	10	1.07 ± 0.07	1.16 ± 0.16	0.10 ± 0.04	9.50 ± 4.76
	15	1.27 ± 0.07	2.04 ± 0.13	0.13 ± 0.04	12.50 ± 8.22
	30	1.32 ± 0.17	2.30 ± 0.11	0.14 ± 0.02	14.90 ± 0.98

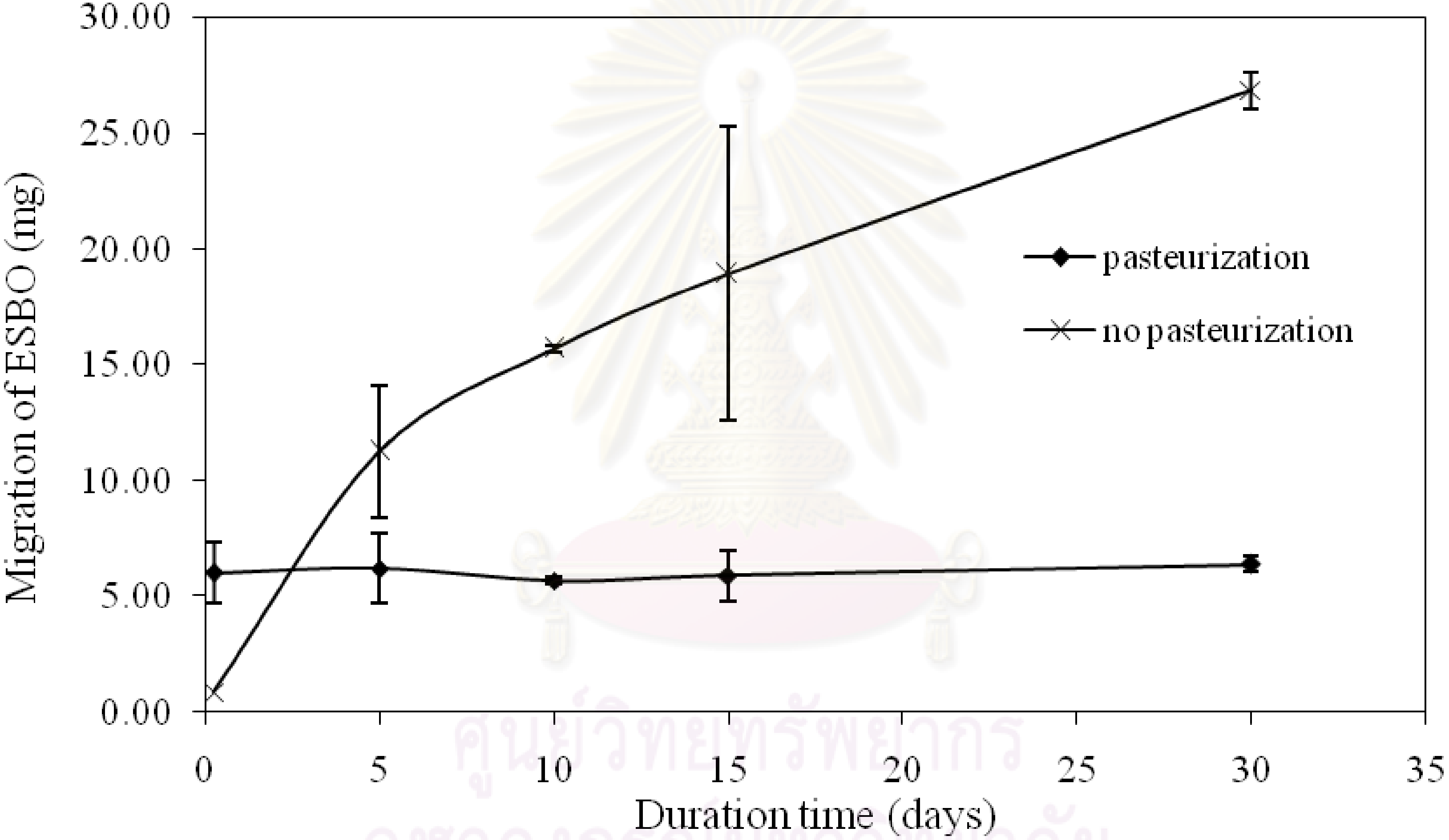


Figure 4.17 The potential migration of ESBO conditioned of initial heating for lug cap 48 mm

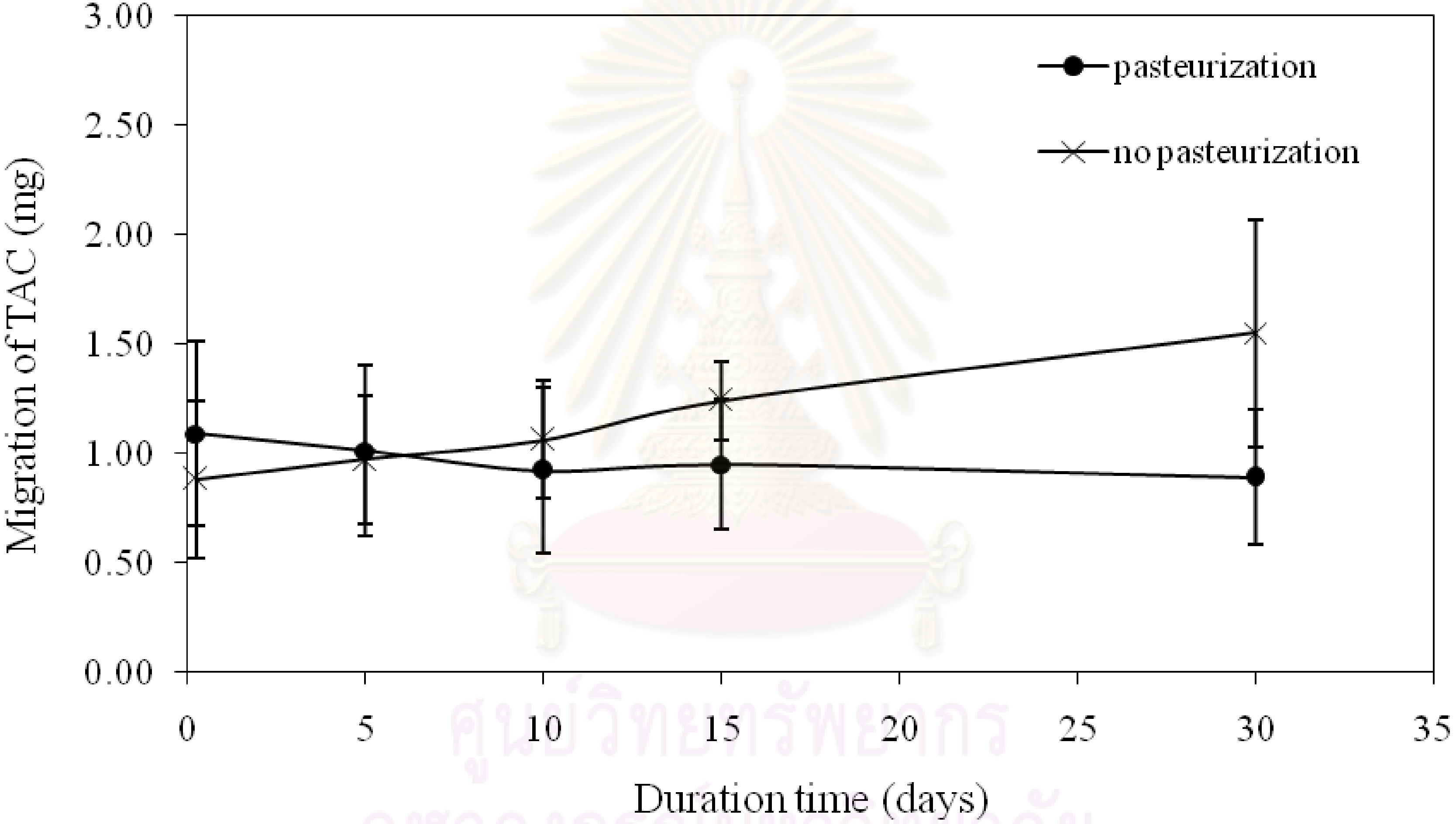


Figure 4.18 The potential migration of TAC conditioned of initial heating for lug cap 48 mm

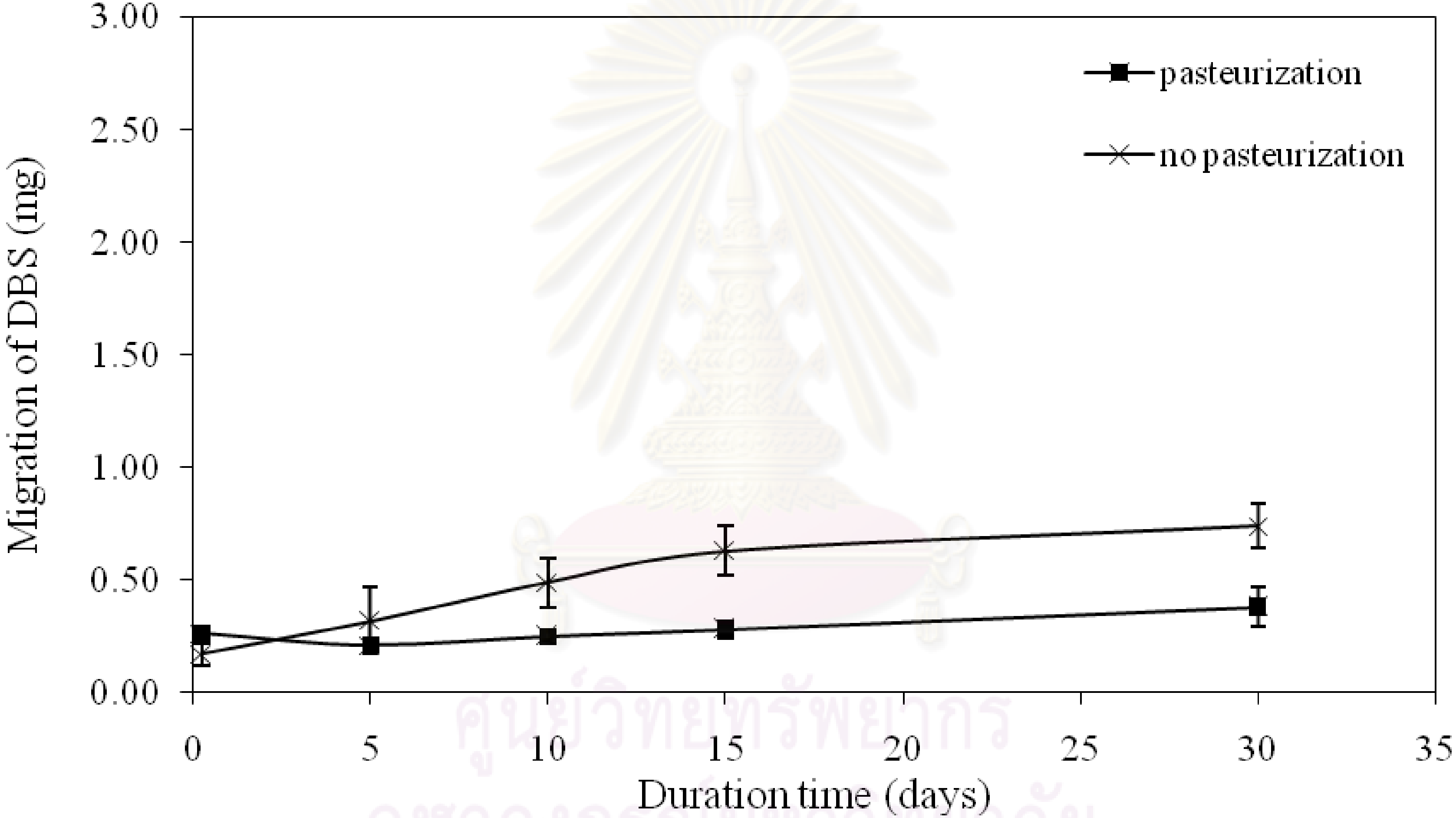


Figure 4.19 The potential migration of DBS conditioned of initial heating for lug cap 48 mm

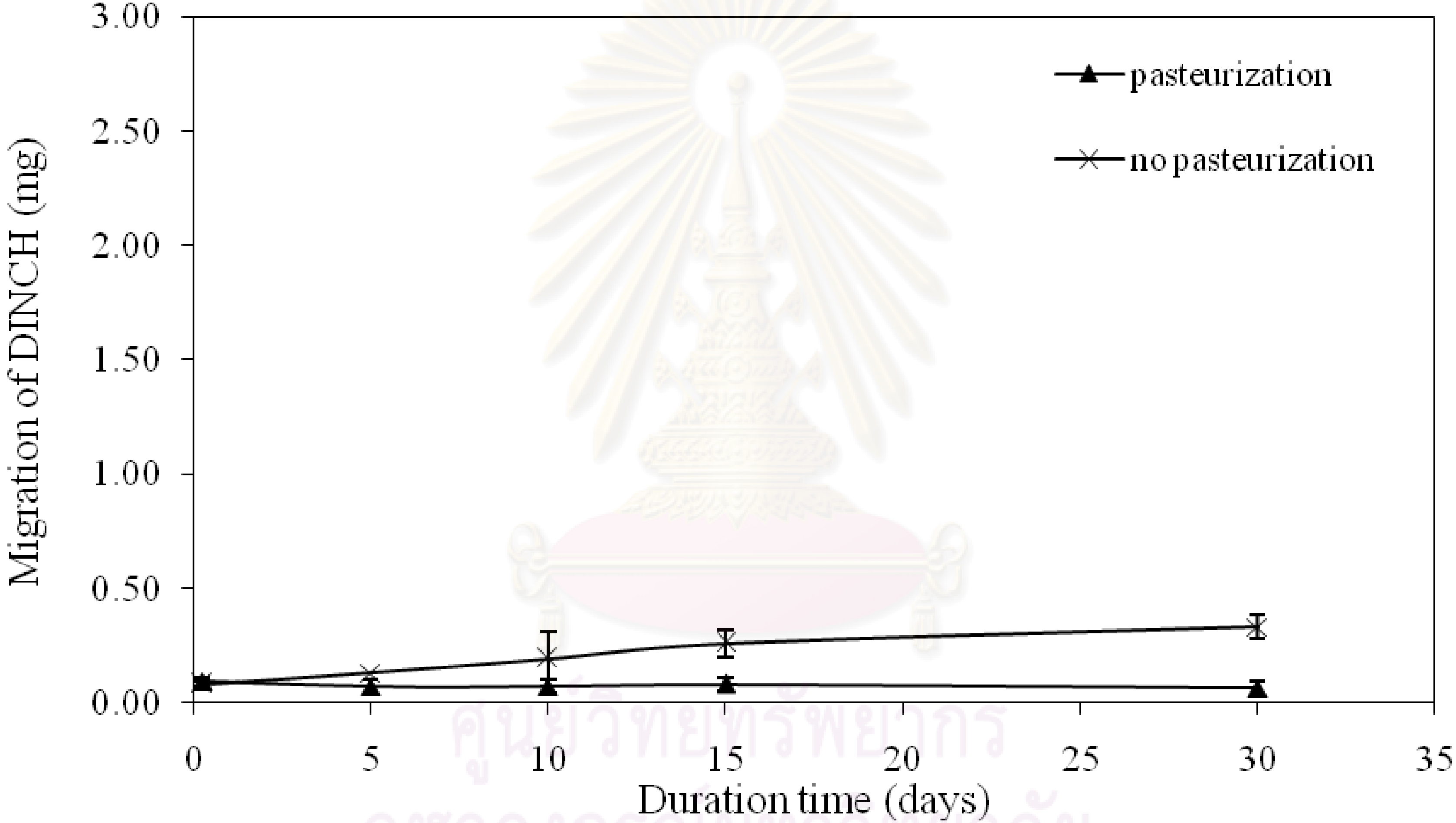


Figure 4.20 The potential migration of DINCH conditioned of initial heating for lug cap 48 mm

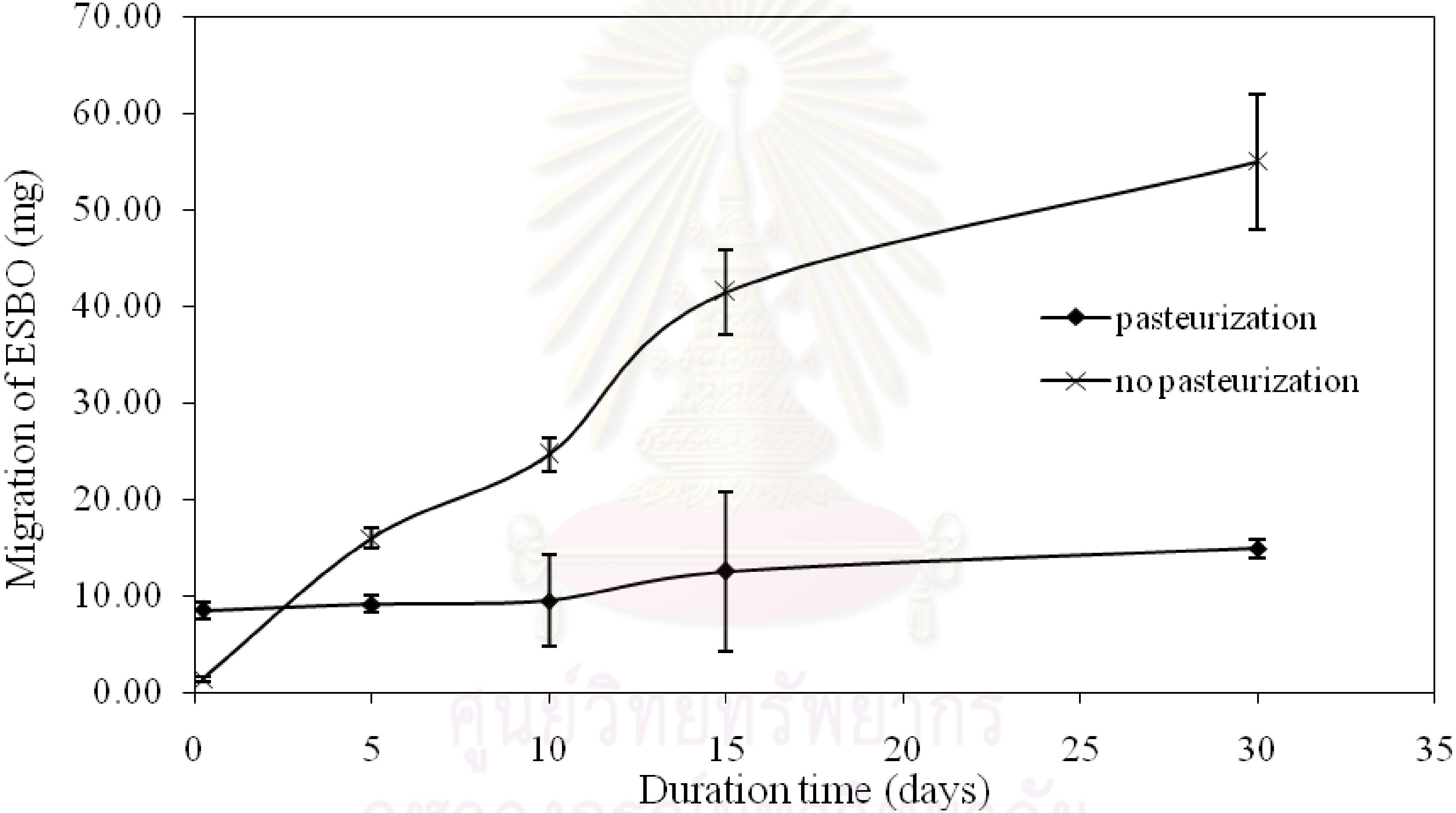


Figure 4.21 The potential migration of ESBO conditioned of initial heating for lug cap 63 mm

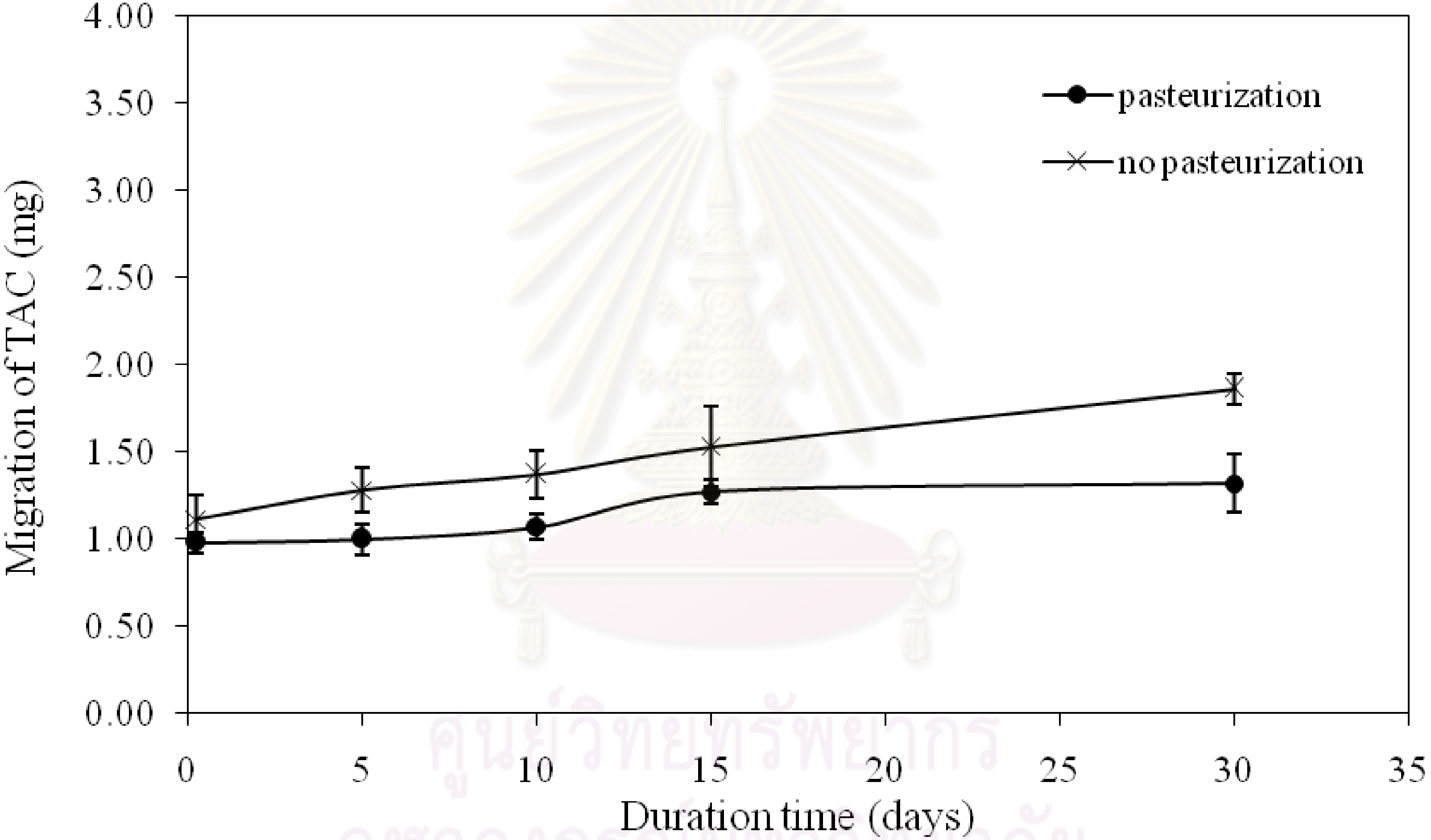


Figure 4.22 The potential migration of TAC conditioned of initial heating for lug cap 63 mm

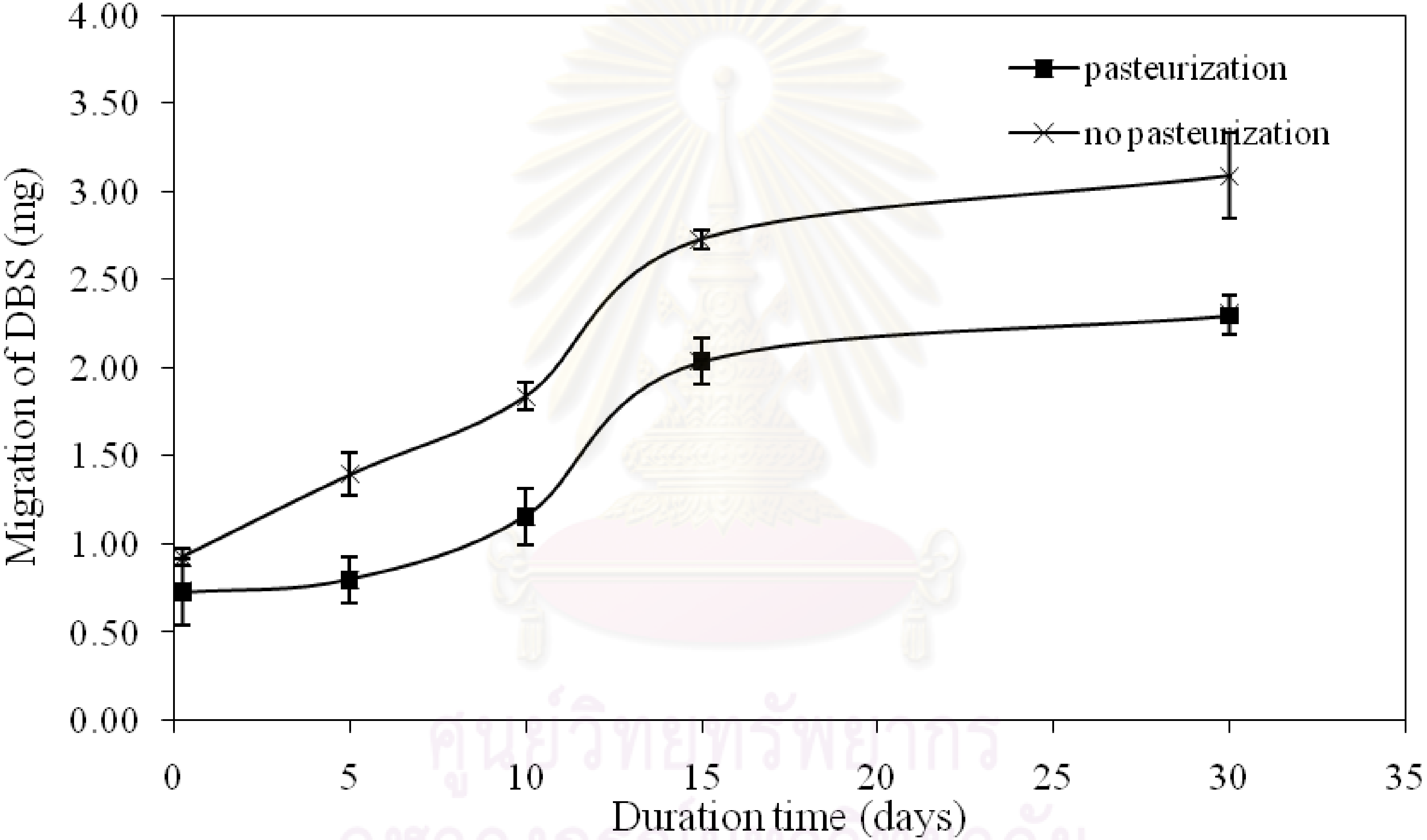


Figure 4.23 The potential migration of DBS conditioned of initial heating for lug cap 63 mm

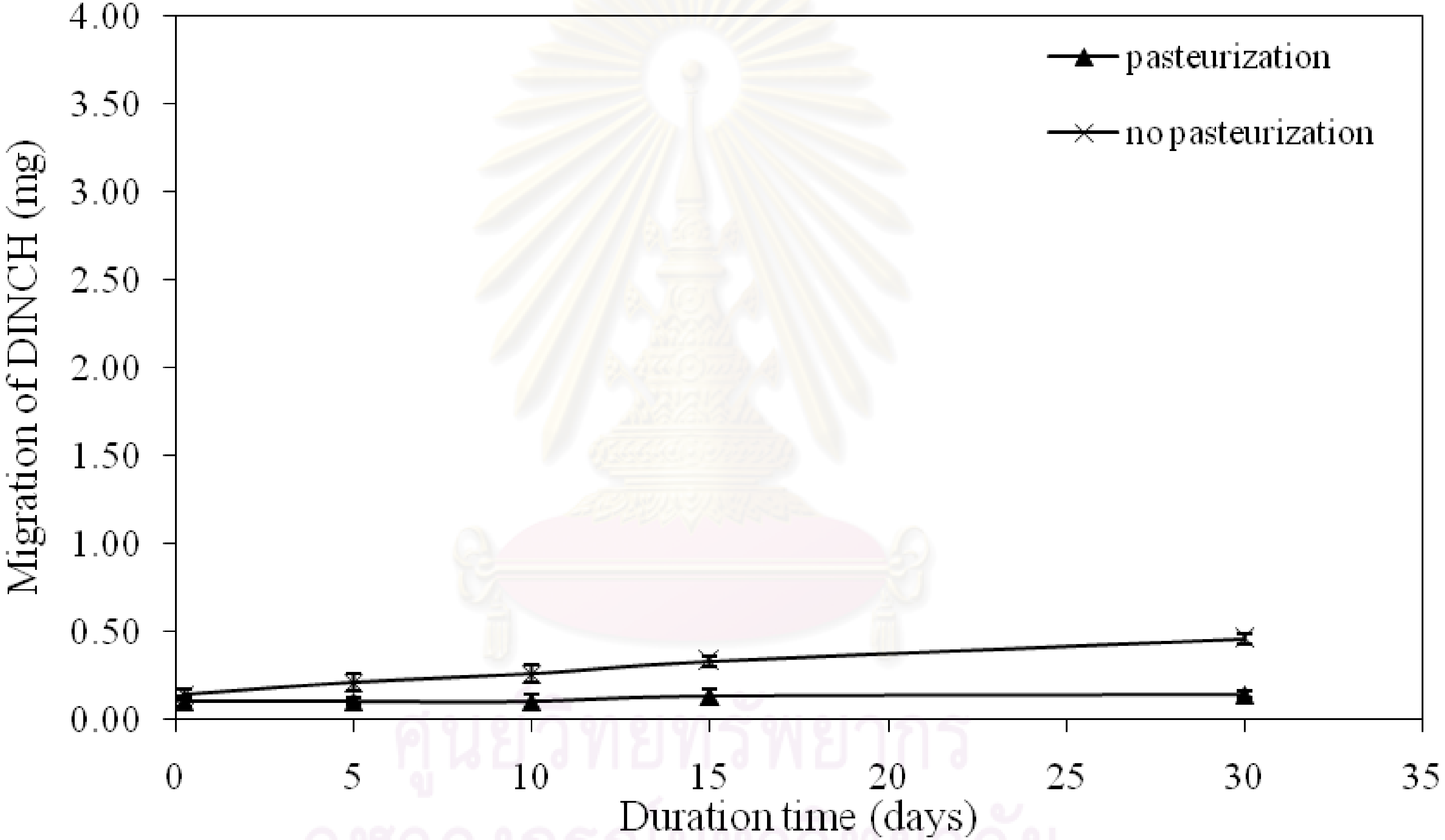


Figure 4.24 The potential migration of DINCH conditioned of initial heating for lug cap 63 mm

4.2.5.5 The Effect of The Contact Surface Area

The influence of the contact surface area on the migration of plasticizers into fatty food simulant was investigated by determination of total amount of plasticizers which migrated from lug cap 48 mm and lug cap 63 mm were compared. The diameter of the lug cap related to the area of the gasket which contacts the fatty food simulant.

The result in Table 4.14 showed the total amount of plasticizers which migrated into fatty food simulant of both lug caps. At room temperature, the migration of plasticizers continuously migrated into fatty food simulant as described in 4.2.4.1. Figure 4.25 showed the large contact area of lug cap 63 mm showed a were higher migration than lug cap 48 mm, as twice in 30 days. Because the high contact area of the gasket made increasing potential transferred of plasticizers to the interface of gasket/fatty food simulant and also increasing extracted and dissolved into fatty food simulant.

Furthermore, the results of high contact temperature showed as increase in the migration of plasticizers into the fatty food simulant and also the migration of plasticizers of lug cap 63 mm were higher than lug cap 48 mm as shown in Figure 4.26. Because the high temperature increased the solubility and mobility of plasticizers to the high contact area of the gasket, therefore the high amount of plasticizers moved easily into fatty food simulant at a higher amount.

On the other hand, the results of the migration of plasticizers into the fatty food with initial heating were invariable compared with the contact time (Figure 4.27). The total amount of plasticizers in the fatty food simulant of lug cap 63 mm was 2.4 times higher than lug cap 48 mm, after 30 days. The invariable migration of plasticizers as described in 4.2.4.3 as well as the high migration of plasticizers of lug cap 63 mm due to the high contact area of gasket as described previously.

However, the percent migration of plasticizers of lug cap 63 mm was lower than the 48 mm lug cap. Due to the ratio of the part of gasket in the food which made contact with the jar content was low, the percent of migration was lower by the significantly larger jar contents. The result showed as that at room temperature, lug cap 63 mm was 36.9% and lug cap 48 mm was 46.9%. The high contact temperature at 60 °C showed the high percent migration of lug cap 63 mm was 83.46% and lug cap 48 mm was 46.8%. Furthermore, the percent migration of initial heating was lower than other parameters at 8.3% and 11.9% for lug cap 63 mm and 48 mm, respectively. The high migration of plasticizers in the jar content depends on the contact surface area and size of food jar. So, the contact surface area had a strong impact to plasticizer migration into fatty food simulant.

Table 4.14 The effect of contact surface area has influence on the migration of plasticizers from lug cap 48 and 63 mm

Migration condition		Amount of migration plasticizer into fatty food simulant (mg)	
		Metal lid 48 mm	Metal lid 63 mm
Room temperature (kept 30 °C)	6 hours	2.00	3.55
	5 days	12.66	18.87
	10 days	17.43	28.07
	15 days	21.05	46.07
	30 days	29.46	60.44
Increasing temperature (duration 10 days)	30 °C	17.43	28.07
	40 °C	31.77	40.73
	60 °C	52.37	76.55
Initial heating (pasteurization and kept at 30 °C)	6 hours	7.68	10.31
	5 days	7.70	11.00
	10 days	7.12	11.83
	15 days	7.18	15.94
	30 days	7.71	18.66

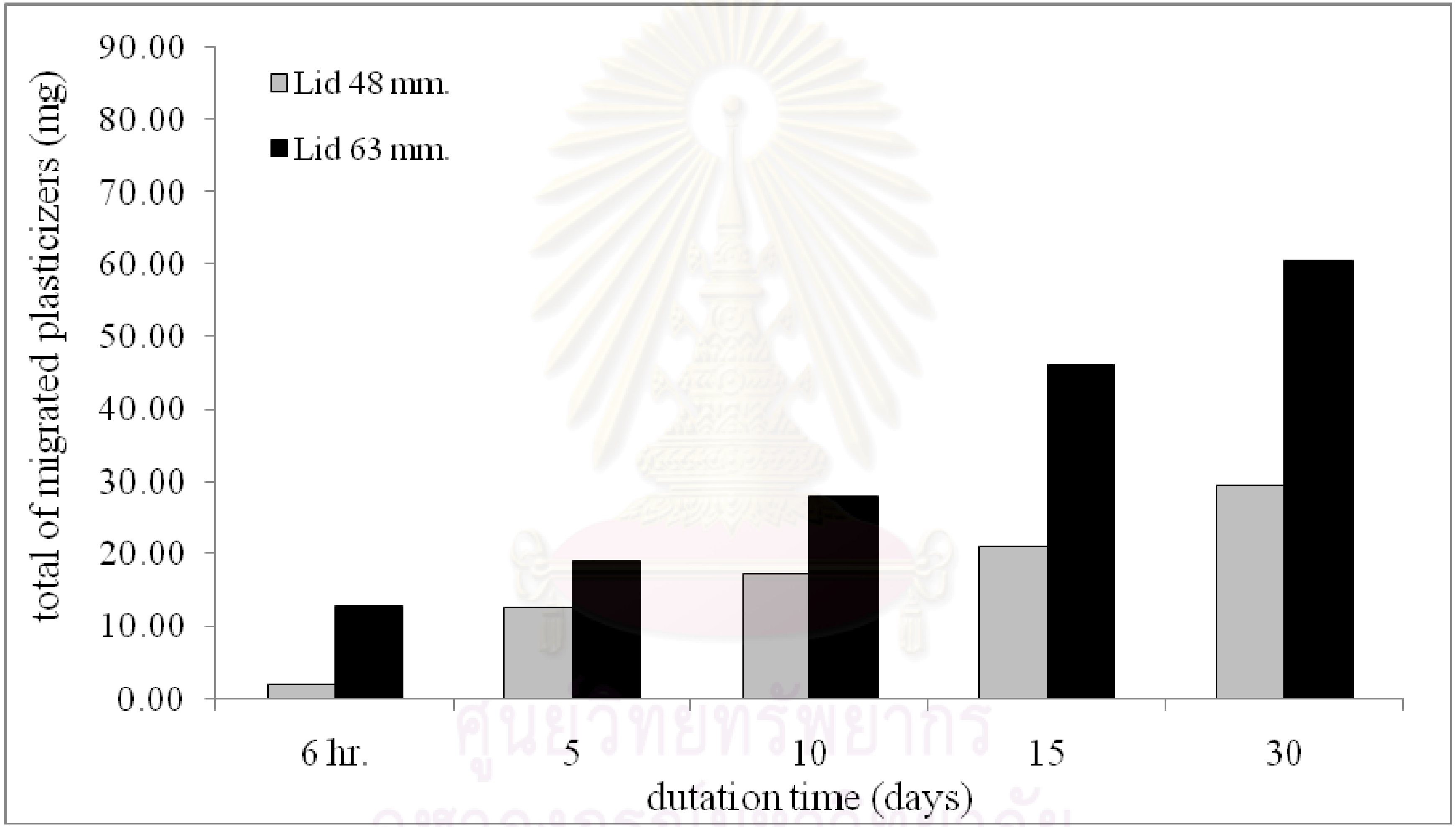


Figure 4.25 The effect of contact surface area has influence on the migration of plasticizers which conditioned of the range of contact time

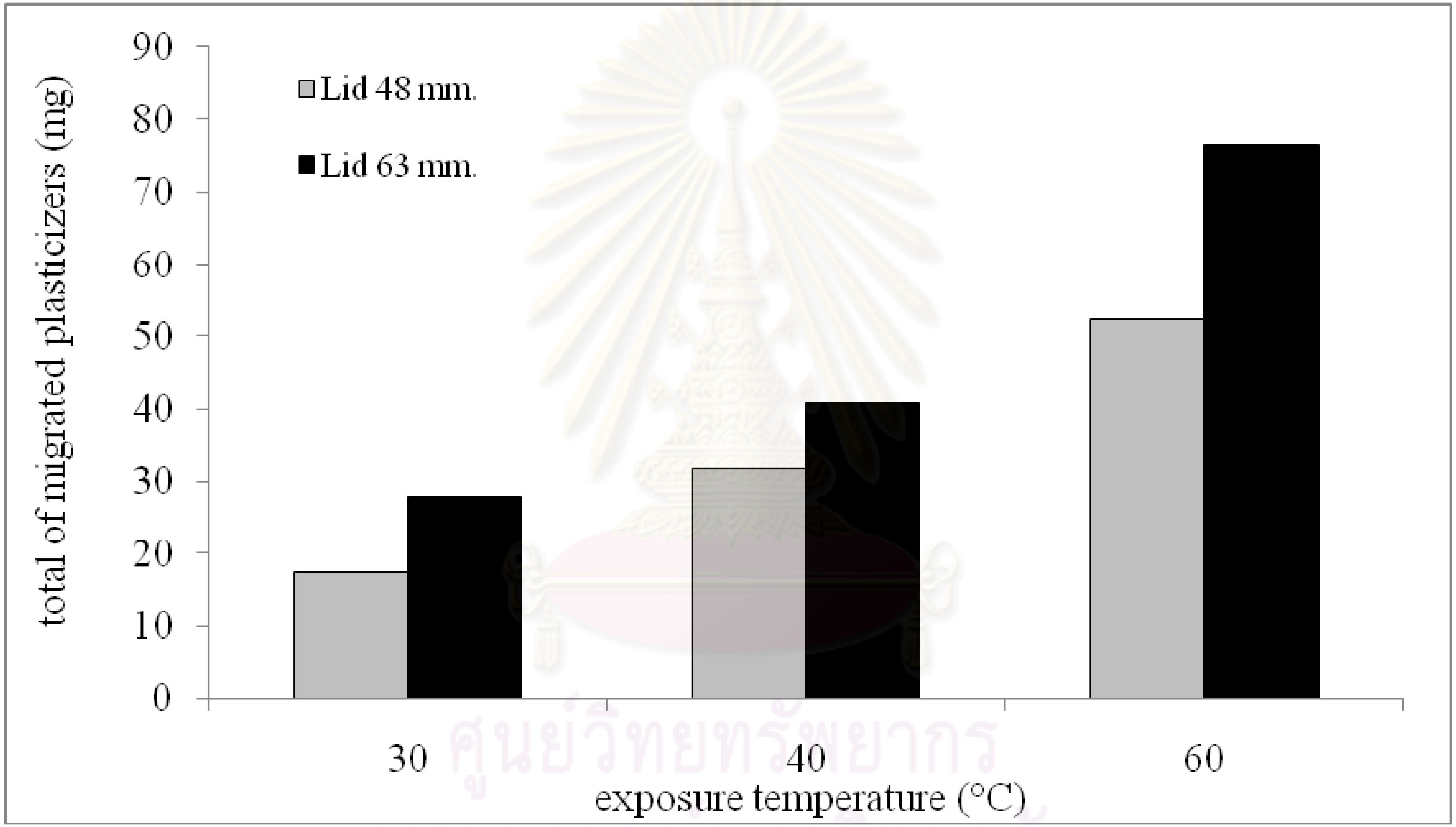


Figure 4.26 The effect of contact surface area has influence on the migration of plasticizers which conditioned of increasing the contact temperature

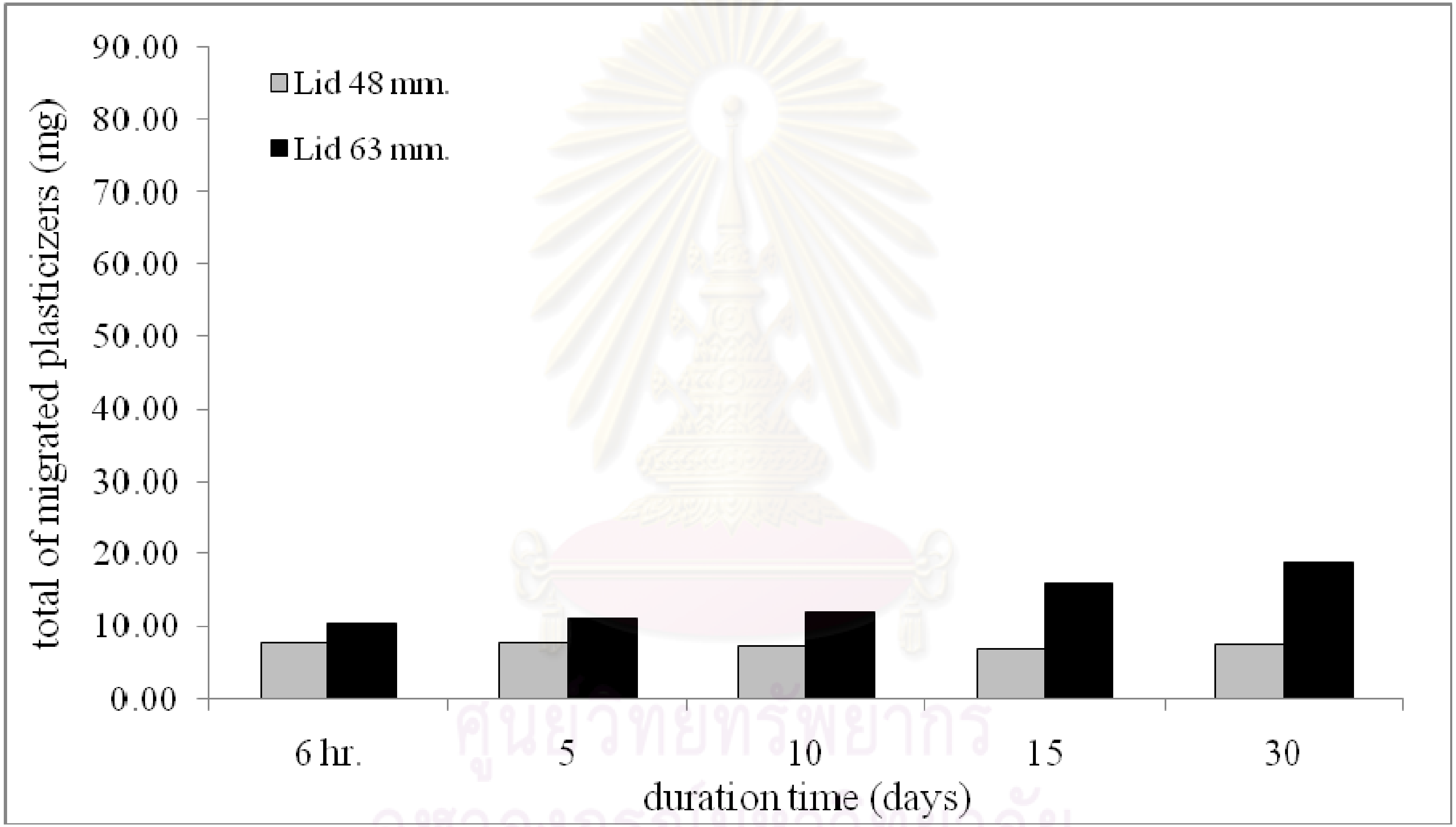


Figure 4.27 The effect of contact surface area has influence on the migration of plasticizers which conditioned of the initial heating

CHAPTER V

CONCLUSIONS AND SUGGECTIONS FOR FURTHER STUDY

In this study, parameters which affect on the potential migration of plasticizers into fatty food (olive oil) were indicated by simulation. Lug cap 48 mm and lug cap 63 mm were identified the components of plasticizers in the gasket prior to migration study. Both techniques of the developed ATR FT-IR microspectroscopy and GC-FID were shown the identical plasticizers such as ESBO, TAC, DBS and DINCH for two types of lug caps.

The ATR FT-IR microspectroscopy with homemade slide-on ATR μIRE accessories was performed in three modes of sampling technique, all ATR spectra were identical. This technique is quite useful for fast qualitative screening. GC-FID was carried out by two methods of analysis such as direct analysis and transesterification by which the advantages of this method were able to confirm the plasticizers in the same extract solutions, resulting robust, less time-consuming, reduce the chemicals and quantity of plasticizers was observed. For the migration studied, the conventional gas chromatography was selected based on qualitative and quantitative information. Total amount of plasticizers in the gasket as 540 ± 14.1 mg and 924.5 ± 105.0 mg for lug cap 48 mm and lug cap 63 mm, respectively.

The simulation for migration test of plasticizers was carried out by the EU standard method. The glass jars contained fatty food simulant (olive oil) and the jars were closured according to the manufacturer produce were studied. The jars were standing up site down and kept them following the studied parameters and further determined the potential migration of plasticizers into fatty food simulants.

The four plasticizers which potentially migrated into the fatty food simulant (olive oil) are TAC, DBS, DINCH and ESBO. The two analysis procedures were directly performed into the fatty food simulants i.e. ESBO analysis procedure by GC-MS using for the analysis of methyl epoxy linoleate of ESBO-derivatized. The chromatograms showed two peaks of diastereoisomers of methyl epoxy linoleate and internal standard and quantify of the ESBO using response factor calculation which corresponded to the concentration in the fatty food simulants. In addition, the monomeric plasticizers analysis procedure by PTV-GC-MS is used for the analysis of the small molecule of plasticizers i.e. TAC, DBS and DINCH. The calibration curves of plasticizers displayed good linearity ($R^2 > 0.9980$) and the relationships of the correlative peak area and the concentration of each plasticizer were used to calculate the concentration of plasticizers in fatty food simulants.

The migration of plasticizers into fatty food simulants from two types of metal lids such as lug cap 48 mm and lug cap 63mm received from the same batch of metal lid productions from manufacture in Thailand were determined. The parameters affected to the migration of plasticizers can be determined from the quantities of plasticizers in the fatty food simulants of each parameter. The plasticizers primarily migrated due to the fatty food simulants coming into contact with the gaskets, and plasticizers were dissolved and migrated into the jar content. The duration time is related to the increase of plasticizers into the foods. At room temperature (30 °C), up to 29.46 and 60.44 mg/lid for lug cap 48 mm and 63 mm of the plasticizers migrate into the fatty food simulant, respectively within 30 days. Typically, ESBO has the highest rate of migration more than 90% into fatty food simulants when compared with all the plasticizers in the gasket. The high temperatures influence the acceleration of the migration of plasticizers. Within 10 days, an increasing in the room temperature of 10 degree Celsius (or 40 °C) caused the higher migration of 1.9 and 1.5 times for lug cap 48 mm and 63 mm, respectively and the higher 20 degree Celsius (at 60 °C) increase the migration of plasticizers to 1.7 and 1.9 times for lug cap 48 mm and 63 mm, respectively. On the other hand, the high temperature from the initial heating had a strong effect on the migration of plasticizers into fatty food simulants. After the initial heating process of pasteurization, the starting point of plasticizers migration was higher than without initial heating as 3.84 and 2.90 times for lug cap 48 mm and 63 mm, respectively. The plasticizers from the jars with initial heating have no increase in migration, contrasting with the jars without initial heating which showed continuous migration into fatty food simulant for 30 days. The last parameter is contact surface area and the migration should be considered at the gasket in food direct contact. The large surface area can increase the proportion of extraction plasticizers by the fatty food simulants leading to the increase of plasticizer migration into fatty food simulations.

Further works should be focused on other, recent finding on plasticizers that are possibly released from the gasket into fatty foods e.g. 2,2,4-trimetyly-1,3- pentandiol diisobutylrate (TXIB) , neopentyl glycol (2-ethylhexanoate), neopentyl glycol 2-ethylhexanoate benzoate and neopentyl glycol dibenzoate.

REFERENCES

[1] European Union. Commission Direction 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs. 2002. Available from: http://ec.europa.eu/food/food/chemicalsafety/foodcontact/2002-72 en. pdf EU 2 [2002, August 6].

[2] European Union. Commission Direction 2004/19/EC amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs. 2004. Available from: http://eur-lex.europa.eu /LexUriServ/LexUriServ.do?uri=OJ:L2004:071:00080:0021:EN:PDF [2004, March 1].

[3] European Union. Commission Direction 2007/19/EC amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food and Council Directive 85/572/EEC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. 2007. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2007L0019:20070420:EN:PDF [2007, April 2].

[4] European Union. Commission Regulation (EC) No 372/2007/EC laying down transitional migration limits for plasticisers in gaskets in lids intended to come into contact with foods. 2007. Available from http://eur-lex.europa.eu /LexUriServ/LexUriServ.do?uri=OJ:L:2007:092:0009:0012:EN:PDF) [2007, April 2].

[5] Biedermann-Brem, S., Biedermann, M., Fiselier, K., and Grob, K. Compositional GC-FID Analysis of Additives to PVC, Focusing on the Gasket of Lids for Glass Jars. Food Addictives and Contaminants 22 (2005): 1274 – 1284.

[6] Fankhauser-Noti, A., Biedermann-Brem, S., and Grob, K. PVC Plasticizers/Additives Migrating from The Gasket of Metal Closures into Food: Swiss Market Survey June 2005. European Food Research and Technology 223 (2006): 447 – 453.

[7] Ežerski, Z., Morkûnas, V., Suman, M., and Simoneau, C. Analytical Screening of Polyadipates and Other Plasticizers in Poly(vinyl chloride) Gasket Seals in Fatty Food by Gas Chromatography-Mass Spectrometry. Analytica Chimica Acta 604 (2007): 29 – 38.

[8] Fankhauser-Noti, A. and Grob, K. Migration of Plasticizers form PVC Gaskets of Lids for Glass Jars into Oily Foods: Amount of Gasket Material in Food Contact, Proportion of Plasticizers Migrating into Food and Compliance Testing by Simulation. Trends in Food Science and Technology 17 (2006): 105 – 112.

[9] Fankhauser-Noti, A., and others. Epoxidized Soy Bean Oil (ESBO) Migrating from The Gaskets of Oil into Food Packed in Glass Jars. European Food Research and Technology 221 (2005): 416 – 422.

[10] Pedersen, G.A., Jensen, L.K., Fankhasen, A., Biedermann, S., Petersen, J.H., and Fabech, B. Migration of Epoxidized Soybean Oil (ESBO) and Phthalates from Twist Closures into Food and Enforcement of The Overall Migration Limit. Food Addictives and Contaminants 25 (2008): 503 – 510.

[11] Fantoni, L., and Simoneau, C. European Survey of Contamination of Homogenized Baby Food by Epoxidized Soybean Oil Migration from Plasticized PVC Gaskets. Food Addictives and Contaminants 20 (2003): 1087 – 1096.

[12] Hammarling, J., Gustavsson, H., Svensson, K., Karlsson, S., and Oskarssom, A. Migration of Epoxidized Soybean Oil from PVC Gasket into Baby Food. Food Addictives and Contaminants 15 (1998): 203 – 208.

[13] Fankhauser-Noti, A., Fiselier, K., Biedermann-Brem, S., Biedermann, M., and Grob, K., Epoxidized Soy Bean Oil Migrating from The gaskets of Lids into Food Packed in Glass Jars Analysis by On-line Liquid Chromatography-Gas Chromatography. Journal of Chromatography A 1082 (2005): 214 – 219.

[14] Suman, M., Tegola, S.L., Catellani, D., and Bersellini, U. Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectroscopy Method for The Determination of Epoxidized Soybean Oil in Food Products. Journal of Agriculture and Food Chemistry 53 (2005): 9879 – 9884.

[15] Biedermann, M., Fiselier, K., and Grob, K. Testing Migration from The PVC Gaskets in Metal Closure into Oily Foods. Trends in Food Science and Technology 19 (2008): 145 – 155.

[16] Castle, L., Sharman M., and Gilbert, J. Gas Chromatographic-Mass Spectrometric Determination of Epoxidized Soybean Oil Contamination of Food by Migration from Plastic Packaging. Journal of AOAC International 71 (1988): 1183 – 1186.

[17] Biedermann-Brem, S., Biedermann, M., Fankhauser-Noti, A., Fiselier, K., Grob, K., and Helling, R. Determination of Epoxidized Soybean Oil (ESBO) in Oily Foods by GC-FID or GC-MS Analysis of Methyl Diepoxy Linoleate. European Food Research and Technology 224 (2007): 309 – 314.

[18] Biedermann, M., Fiselier, K., and Grob, K. Injector-Internal Thermal Desorption form Edible Oils. Part 1: Visual Experiments on Sample Deposition on The Liner Wall. Journal of Separation Science 28 (2005): 1550 – 1557.

[19] Fiselier, K., Biedermann, M., and Grob, K. Injector-Internal Thermal Desorption form Edible Oils. Part 2: Chromatographic Optimization for The Analysis of Migrants form Food Packaging Material. Journal of Separation Science 28 (2005): 2144 – 2152.

[20] Fankhauser-Noti, A., and Grob, K. Injector-Internal Thermal Desorption form Edible Oils Performed by Programmed Temperature Vaporizing (PTV) Injection. Journal of Separation Science 29 (2006): 2365 – 2374.

[21] Piringer, O-G., and Baner, A.L. Plastic Packaging Materials for Food. Germany: Wiley-VCH, 2000.

[22] Larousse, J., and Brown, B. E. Food Canning Technology. New York: Wiley-VCH, 1997.

[23] Bureau, G., and Multon, J-L. Food Packaging Technology volume 1. New York: WILEY-VCH, 2007.

[24] Pocas, M der F., and Hogg, T. Exposure Assessment of Chemicals form Packaging Materials in Foods A Reviews. Trends in Food Science & Technology 18 (2007): 219 –230.

[25] Rees, J.A.G., and Bettison, J. Processing and Packaging of Heat Preserved Foods. New York: AVI, 2007.

[26] Biedermann-Brem, S., Biedermann, M., and Grob, K. Reaction of Epoxidized Soya Bean Oil (ESBO) with Hydrogen Chloride Formed in PVC: Investigation on Model Systems. Mitteilungen aus Lebensmitteluntersuchung und Hygiene 95 (2004): 261 –280.

[27] Lau, O-W., Wong, S-K. (Review) Contamination of Food From Packaging Material. Journal of Chromatography A 882 (2000): 255 – 270.

[28] Barnes, K., Sinclair, C.R., and Watson, D.H. Chemical Migration and Food Contact Materials. Boston: CRC, 2007.

[29] Arvanitoyannis, I.S., and Bosnea, L. Migration of Substances from Food Packaging Materials to Foods. Critical Review in Food Science and Nutrition 44 (2004): 63 – 76.

[30] Ekgasit, S., Vongsvivut, J., and Thongnopkun, P. ATR FT-IR Absorption Enhancement of a Thin Film under the Photon-Tunneling Condition. Analytical Sciences 23 (2006): 847 – 851.

[31] Thongnopkun, P., and Ekgasit, S. Attenuated Total Reflection Fourier Transform Infrared Spectra of Faced Diamonds. Analytica Chimica Acta 576 (2006): 130 – 135.

[32] Urban, M. W. Attenuated Total Reflectance Spectroscopy of Polymer: Theory and Practice. Washington DC: American Chemical Society, 1996.

[33] Calmers, J. M., and Griffith, P. R. Hand book of Vibrational Spectroscopy Volume 2. United Kingdom: John Wiely & Sons, 2002.

[34] Harrick. N. J. Internal Reflection Spectroscopy. New York: Herrick Scientific Corporation, 1979.

[35] Wilhelm, P. Applications of FT-IR Microscopy with Materials Analyses. Micron 27 (1996): 314 – 344.

[36] Ekgasit, S., and Padermshock, A. Optical Contact in ATR/FT-IR Spectroscopy. Applied Spectroscopy 55 (2002): 1352 – 1359.

[37] Buffeteau, T., Desbat, B., and Eyquem, D. Attenuated Total Reflection Fourier Transform Infrared Microspectroscopy: Theory and Application to Polymer Samples. Vibrational Spectroscopy 11 (1996): 29 – 36.

[38] Ekgasit, S., Vongsvivut, J. and Thongnopkun, P. A Novel ATR FT-IR Microspectroscopy Technique for Surface Contamination Analysis without Interference of the Substrate. Analytical Sciences 23 (2006): 863 – 868.

[39] Katon, J. E. Infrared Microspectroscopy. A Review of Fundamentals and Applications. Micron 27 (1996): 303 – 314.

[40] Feigenbaum, A., and others. Functional Barriers: Properties and Evaluation. Food Addictives and Contaminants 22 (2005): 956 – 967.

APPENDICES

APPENDIX A

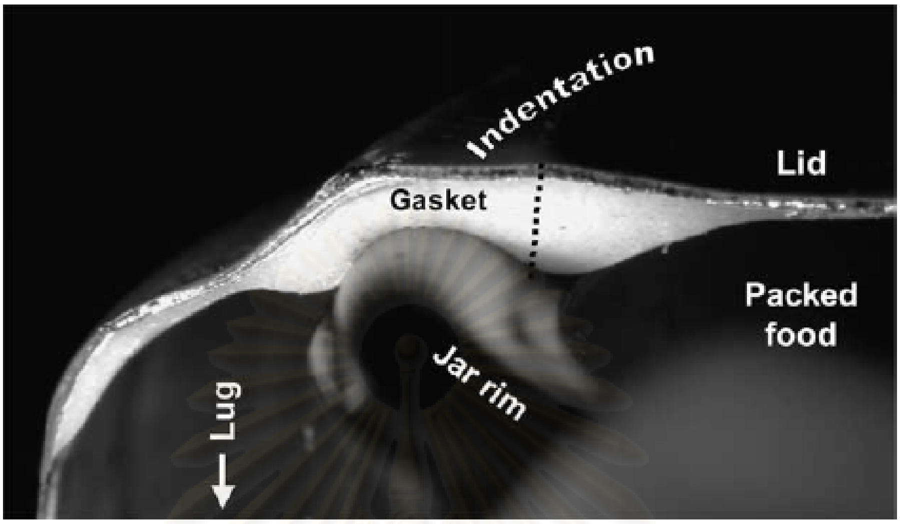


Figure A-1 The cutaway of lug cap on the rim of glass jar, on the right hand is part of packed food intend to come to direct contact with gasket and a white sheet as the gasket which present two bulges from the indentation between metal lid and the gasket.

APPENDIX B

Figure B-1 The ATR FT-IR microspectrometer with homemade slide-on Ge μATR accessory. (A) Ge housing, (B) the slide-on Ge μATR, (C) Ge μATR accessory.

APPENDIX C

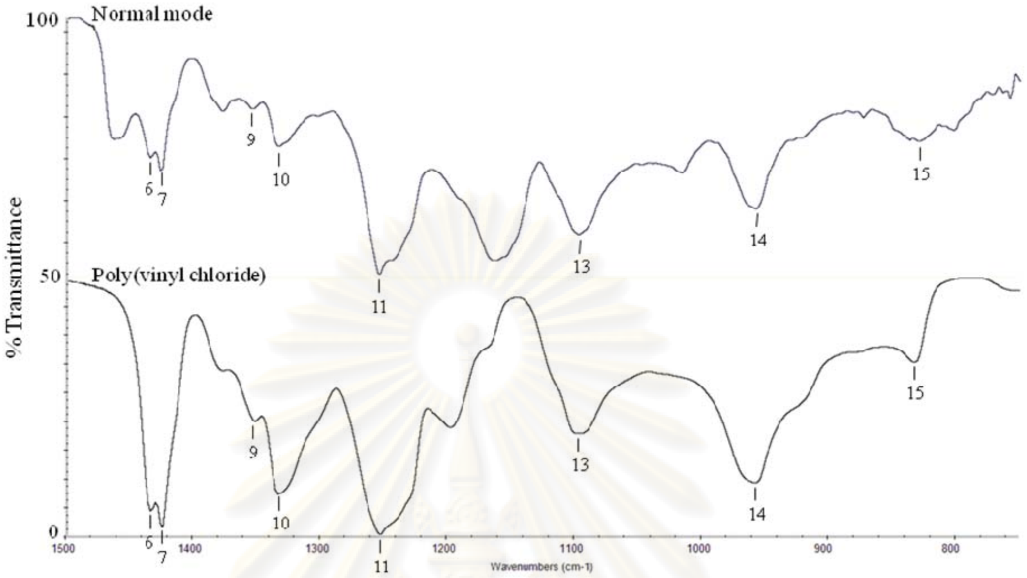


Figure C-1 ATR spectrum of normal mode at 1,500-750 cm^{-1} associated the spectrum of PVC gasket

APPENDIX D

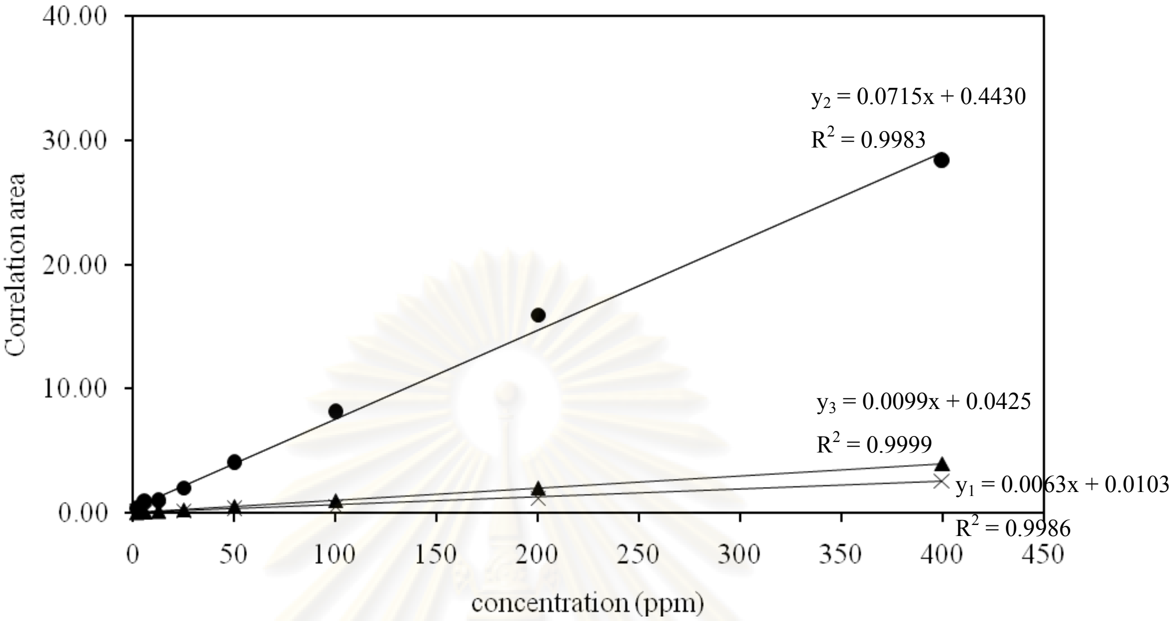


Figure D-1 Calibration curve of TAC (n = 1-3) by GC condition listed in Table 4.7

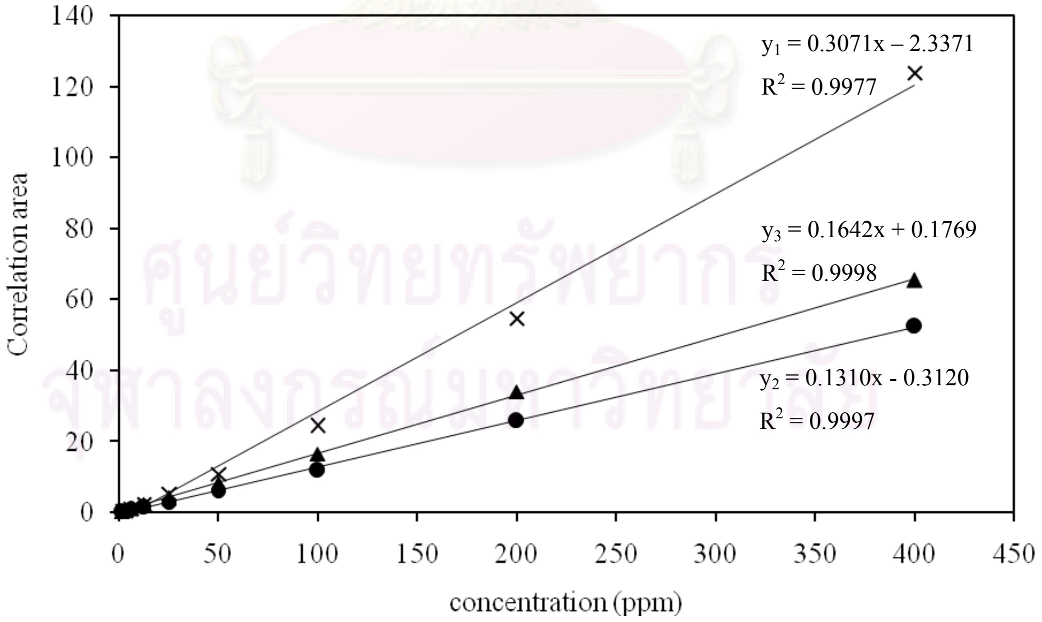


Figure D-2 Calibration curve of DBS (n = 1-3) by GC condition listed in Table 4.7

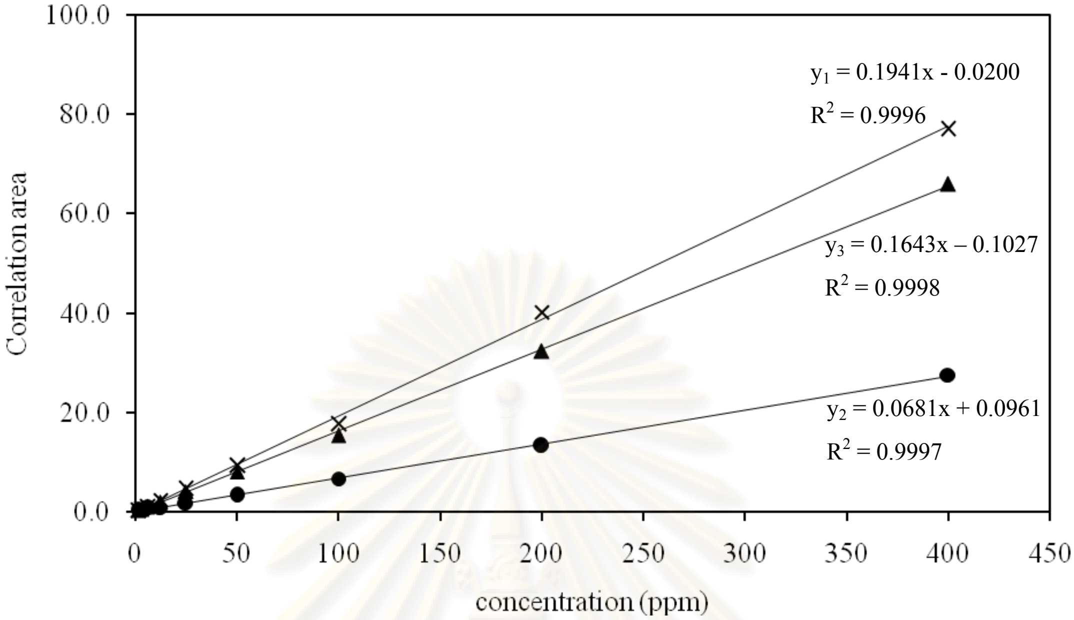


Figure D-3 Calibration curve of DBS (n = 1-3) by GC condition listed in Table 4.7

VITA

Miss Chanchira Chanprasert was born on March 26, 1984, in Pattani, Thailand. She received a Bachelor Science (Education) Degree in Chemistry (second class honors) from the faculty of Education, Prince of Songkla University in 2005. After that, she continued her graduate work in Analytical Chemistry, concentration in chromatography and separation, at the Department of Chemistry, Faculty of Science, Chulalongkorn University. And will receive her Master of Science Degree in July 2009.